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Photo, Walter Stoneman]

R. G. Hatton, C.B.E., M.A., D.Sc., F.R.S., V.M.H.

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R. G. HATTON

An Appreciation

On the completion of Volume XXIII of the *Journal of Pomology and Horticultural Science*, Dr. R. G. Hatton felt obliged by the pressure of other work to relinquish the post of Joint Editor which he had held for more than twenty years.

Dr. Hatton's connection with the *Journal* has been continuous from its start. To its first volume he contributed his noteworthy paper on "Black Currants, A Method of Classification." In this he described the work on that subject at East Malling which established the grouping, by botanical characters of the four main classes, of the varieties of that fruit and paved the way to the correct nomenclature of the individual varieties.

Apart from this and other later contributions from his own pen the long sequence of the numerous papers from other workers at his Station, which have owed so much to his inspiration, has served a major part in earning for the *Journal* the high world-wide repute which it has gained as the leading publication for the presentation of British horticultural research.

He first became officially connected with the *Journal* in 1922, when, under its original title of the *Journal of Pomology*, it was taken over from its founder, Mr. E. A. Bunyard, after the issue of Volumes I and II. Until then it had been produced and financed by the latter, who initiated it from a desire to lay before English fruitgrowers the results of research beginning to flow from the two organized English fruit research stations, East Malling and Long Ashton, then in their infancy, from other horticultural research centres and from individual workers in the country. At that time the number of subscribers was small, the majority of them being practical fruitgrowers and firms directly connected with the industry.

With its limited circulation it was impossible at that stage to produce it without financial loss. Hence the Ministry of Agriculture, recognizing the potential scope and value of the *Journal*, approved an arrangement under which the two Research Stations mentioned, with the addition of the Horticultural Institute at Cambridge University, assumed responsibility for its future publication and finance. A small Publication Committee, composed of the Directors of these three Research Centres, two representatives of the Ministry and Mr. Bunyard, was established; the latter continued, for the two following years, to act as Editor.

In view of the wider scope envisaged for the *Journal*, its title was expanded to that of the *Journal of Pomology and Horticultural Science.* This change was accepted with some reluctance by the Editor, who feared that the original objects might be obscured by the broader range of the new title and the support of the practical fruitgrower be lost through a possible predominance of contributions more appropriate for study by research workers. On the other hand the Research Stations stressed the need for an authoritative journal in which could be presented the results of their investigations to supplement the necessarily briefer and more incomplete progress accounts of the work which appeared in their own Annual Reports. In effecting this change the influence of Dr. Hatton was a decisive factor; for it was realized that the interests of the practical fruitgrower would be fully secured in any undertaking with which he was associated. In fact, to him more than to any other one person, the credit is due both for the survival of the *Journal* through those difficult early years and for the world-wide circulation which it has subsequently attained.

In 1925 it became necessary to make new editorial arrangements and the personnel of the Publication Committee was increased. At the end of that year Dr. Hatton became Acting Editor and a year later was joined in that capacity by the Director of the Long Ashton Research Station. From that time to the present it has been an accepted arrangement that the editorship of the *Journal* should be in the hands of the Directors of the two fruit research stations, while the Publications Committee has been strengthened from time to time to make it more representative of the constantly expanding range of horticultural research.

Recognition of that expansion, of the concurrent increase in the subjects dealt with in the *Journal* and of its ever widening circulation was expressed in 1936, when a series of important changes was made. In consequence of its circulation in thirty-eight countries outside the British Isles, four associate editors representing Australia, Canada, New Zealand and the Crown Colonies were added to the Editorial Board, while the late Dr. G. H. Pethybridge was appointed Assistant Editor and the two Acting Joint Editors became Joint Editors. A further recognition of its widening scope occurred as recently as the issue of the first number of Volume XXIV, when it appeared under its abbreviated title of the *Journal of Horticultural Science.*

Such have been the stages in the evolution of the *Journal* to its present important position in the literature of scientific Horticulture and with which our retiring Editor has been so intimately associated from the beginning. Only those who have been his colleagues in the editorship and on the Publication Committee can fully appreciate the extent to which its success has been due to his unceasing interest and endeavour.

His services in this respect are the more remarkable when it is remembered that throughout his editorship he was Director of East Malling Research Station, with ever-expanding duties and responsibilities, and was also a frequent contributor of articles embodying the results of his own important researches.

At the end of 1948 Dr. Hatton retired from the Directorship of East Malling Research Station; his achievements in that office will be commemorated elsewhere. Very fortunately he remains on the Publication Committee of the *Journal of Horticultural Science,* where we hope to have the benefit of his wide experience and wise judgement for many years to come. B.T.P.B.

The announcement that H.M. the King would confer upon Dr. Hatton the honour of knighthood was made after Professor Barker's appreciation had been set up in type. The Editors have much pleasure in being able to add so gratifying a postscript as the Journal goes to press.

THE VARIABILITY OF APPLE TREES

I. THE EXTENT OF CROP VARIATION AND ITS MINIMIZATION BY STATISTICAL MEANS

By S. C. PEARCE
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When planning an experiment, a necessary preliminary is to enquire into the extent of variability in the trees. If they are very different one from another it may be better not to use them for experimental purposes at all because it is quite likely that any differences introduced by the treatments will not show up among the differences already present. On the other hand, if they are very uniform it may be possible successfully to conduct an experiment using fewer than usual.

The attention given in recent years to the standardization of pomological plant material has been most necessary from the point of view of the experimenter, to whom uniformity of material is as important as uniformity of land. After so much effort, it seems worth while to examine the present position in respect of uniformity and, to that end, this paper sets out the variability of four sets of apple trees at East Malling. It is hoped that other workers will publish surveys of the variabilities they encounter because, obviously, little should be inferred from results obtained in only one set of ecological conditions.

In this paper attention has been concentrated on cropping figures. Similar work has already been done on growth records and it appears that these often give results quite different from those here presented.

The following is an enquiry into the variability of apple crops at different times in the life of the plantation, assuming that an experiment is to be continued for a range of periods. It also explains how useful reductions in variability may be brought about by taking into account such quantities as the size of each tree at the beginning of the experiment and the amount of crop it has already borne.

MATERIAL.

Trial I, which has been described elsewhere (Hoblyn, 1931, 1941), consisted of eight randomized blocks of eight manurial treatments, four with potash and four without. Because of the large difference due to potash, the data were considered as coming from two trials, IA comprising trees receiving potash and IB comprising the rest. Each plot contained 25 trees, so the blocks were extremely large being 200 feet long and 100 feet wide ; only one tree was, however, considered in each plot, namely a Cox's Orange Pippin on M.I. These trees were planted at two years of age.

Trial II had been planted in the first place as a commercial plantation of Cox's Orange Pippin on M.IX with Worcester Pearmain pollinators. Ten blocks were used, each containing six trees of the main variety and each 36 feet long by 24 feet wide.

Trial III was one of the classical East Malling rootstock trials, now nearly 30 years old. Four blocks were taken, each 60 feet long by 30 feet wide and containing eight trees. In each block there were six trees of Lane's Prince Albert on M.XII, these alone being considered.

Trial IV was part of a plantation used for pruning experiments planted on the so-called " Free " stocks that were common before the standardization of clones. Planting took place in the spring of 1914. The variety was Worcester Pearmain, which is more regular in cropping than the others being considered. Each block consisted of four trees that had been pruned alike, occupying an area of 112 feet by 18 feet. These trees are included chiefly to show the improvement in variability brought about by using clonal rootstocks.

The four trials thus cover a wide range of vigour in the rootstocks they contain and, what is more, they comprise three plantations planted for experimental purposes and one for commercial, while Trial I had exceptionally large blocks. They are in fact very different from one another and, between them, they represent most examples that could arise in practice.

MEASURE OF VARIATION.

For Trials IA and IB, variation was measured by the ordinary standard error of the randomized block design expressed as a percentage of the mean, i.e., by the coefficient of variability. In Trials II, III and IV, where there were no differential treatments to give effects needing eliminations, it was sufficient to express the " within blocks " variation in the same way. Throughout the Tables, coefficients of variability in excess of 30 per cent. are distinguished by black type as being unduly high. With material as variable as this, it would be extremely difficult to design a wholly satisfactory experiment.

VARIATION.

Initial Periods. In many trials the treatments are applied from the beginning and attention is therefore directed to the total crop during the opening years of life. In Table I are set out the variabilities of crops from the trials for the first 5, 7, 9, 11, 13, 15, and 17 years from budding. It appears, as would be expected, that the older the trees the more uniform is the total crop. For Trials IA, IB and II this effect seems to have been arrested after eleven years or so, but the variability of Trials III and IV was still falling at 17 years. There is a suggestion that the more dwarfing the rootstock the sooner variability settles down.

TABLE I.

Coefficients of Variability of Crop during Initial Periods.

Period	Trial				
	IA Cox/I. High K.	IB Cox/I. Low K.	II Cox/IX.	III Lane's/XII.	IV Worcester/ Free.
0– 5 years	—	—	**47**	—	—
0– 7 ,,	**47**	**44**	**43**	**67**	**151**
0– 9 ,,	**39**	**41**	28	**38**	**42**
0–11 ,,	30	27	27	23	**35**
0–13 ,,	25	24	28	21	**32**
0–15 ,,	30	29	—	16	30
0–17 ,,	**31**	26	—	14	27

Later Periods. In some other trials, by contrast, the trees are planted initially without any differential treatments and it then becomes important to enquire what variability may be expected in a later period, i.e. one in which the crops are measured not from planting but from some later date. Table II, columns U2, U4 and U6, sets out some information on this point. It should, perhaps, be explained that by, for example, " crops in a two-year period after the application of treatments in the fifth year " is meant the combined crops of the sixth and seventh years and similarly in all the other instances. It will be seen that in general the trees were becoming more uniform as they got older and also, as with the initial periods, the longer the experiment was continued the more uniform were the total crops obtained; both rules, however, have many exceptions.

ADJUSTMENTS OF RESULTS.

Initial Periods. It might be objected that any conclusions drawn from Tables I and II, columns U, are quite misleading because it is not good statistical practice to interpret figures without, wherever possible, making adjustments to allow for measureable sources of variation. Thus, for an initial period there is available the method of Papadakis (1937, see also Bartlett, 1938). This worker suggested that the result from one tree or plot should be judged in relation to the performance of its neighbours, i.e. a high yielding tree should not be taken to indicate that it is receiving a beneficial treatment if all its neighbours are yielding well also; on the other hand, special weight should be attached to it if the trees round about are doing badly. He therefore suggested an adjustment to be applied to the result from each plot or tree which will here be referred to as Adjustment A. Again, it may be that the variation in crops over an initial period may be largely eliminated by a covariance correction upon the sum of maiden height and the length of extension growth made between the maiden year and planting (Adjustment B) or upon trunk girth at planting (Adjustment C). The data of Trial II (Cox on IX) were suitable for the use of Adjustment A and of Trials IA and IB (Cox on I) for Adjustments B and C. Although the variations after the application of these adjustments were all worked out, they are not presented in any Table, because none of the adjustments proved to be of the least use in reducing variation below the figures shown in Table I. At the present time, no statistical means are known to the writer of eliminating or reducing the variation of crops during an initial period.

Later Periods. Adjacent trees (A) and initial girth (C) may be used for later periods as easily as for initial ones except, of course, that in C the adjustment is made on the basis of trunk girth at the time of applying the treatments (or, better, during the dormant period preceding, if the treatments are applied during the growing season). Extension Growth (B) is unlikely to be of much use because of the labour needed to measure the total growth of any but the youngest and smallest trees, so it has been ignored in this section. Two further adjustments, D and E, have been considered instead. D is a covariance correction upon the area of cross-section of trunk at the time of application of treatments, this measurement being easily obtainable from the trunk girth. E is a covariance correction upon the total crop in an initial period ending with the application of the treatments. Trunk area of cross-section (D) was not considered to be of sufficient interest for it to be tried on more than one trial, namely, III (Lane's on XII). Only Trial II, Cox on IX, was suitable for adjustment by adjacent trees (A).

Adjustment by area of cross-section (D) gave variabilities no lower than adjustment by girth (C) and consequently did not repay the extra work involved. Adjustment by adjacent trees (A) again gave no useful reduction in variability and its use is not recommended. As to adjustment by girth (C) and by previous crops (E) these did appear promising and the results are set out in Table II for all trials except IV (Worcester on Free stocks). For Trials IA and IB (Cox on I) neither was very good, the previous crops giving slightly the better results but for the other two trials the girth was decidedly to be preferred. It is not known what determines the value of either adjustment, so only a general recommendation is possible, and this is to correct upon girth (C) rather than upon previous crops (E). Sometimes the previous crops would in fact be better and sometimes the correction upon girth will do little good, but if the above recommendation is consistently followed the effect should be beneficial.

TABLE II.

Coefficients of Variability for Crop during later Periods with and without Adjustment.

Trial.	Treatments Applied.	Adjustment and length of period.								
		U2	U4	U6	C2	C4	C6	E2	E4	E6
IA (Cox/I, High K)	7th Year ..	**45**	**32**	26	**46**[0]	**32**[0]	26[0]	**44**[0]	**31**[0]	27[0]
	9th ,, ..	26	25	**32**	26[0]	21[1]	27[2]	22[2]	23[1]	28[1]
	11th ,, ..	29	**35**	**35**	21[3]	26[3]	25[3]	27[1]	28[2]	29[2]
	13th ,, ..	**46**	**40**	—	**37**[2]	29[3]	—	**34**[3]	30[3]	—
	15th ,, ..	**36**	—	—	**35**[0]	—	—	24[3]	—	—
IB (Cox/I, Low K)	7th Year ..	**58**	30	26	**56**[0]	30[0]	26[0]	**56**[0]	30[0]	26[0]
	9th ,, ..	30	25	30	30[0]	25[0]	**31**[0]	30[0]	24[0]	28[0]
	11th ,, ..	26	**34**	30	24[1]	**31**[1]	29[0]	22[2]	25[3]	24[2]
	13th ,, ..	**42**	**34**	—	**39**[1]	**32**[0]	—	20[3]	27[2]	—
	15th ,, ..	29	—	—	28[1]	—	—	24[2]	—	—
II (Cox/IX)	5th Year ..	**96**	30	28	**97**[0]	25[3]	21[3]	**97**[0]	30[0]	27[1]
	7th ,, ..	**31**	28	29	25[3]	18[3]	19[3]	30[0]	27[1]	28[0]
	9th ,, ..	**31**	**31**	—	20[3]	19[3]	—	24[3]	24[3]	—
	11th ,, ..	**34**	—	—	22[3]	—	—	22[3]	—	—
III (Lane's/XII)	7th Year ..	**40**	23	21	**41**[0]	19[2]	17[2]	**40**[0]	23[0]	21[0]
	9th ,, ..	23	21	16	19[2]	17[2]	12[3]	19[2]	18[1]	16[0]
	11th ,, ..	24	17	15	20[2]	13[3]	11[3]	21[2]	17[0]	15[0]
	13th ,, ..	19	16	—	14[3]	11[3]	—	18[0]	16[0]	—
	15th ,, ..	16	—	—	12[3]	—	—	14[1]	—	—
IV (Worcester/ Free)	7th Year ..	**42**	**35**	**32**	—	—	—	—	—	—
	9th ,, ..	**38**	**33**	**31**	—	—	—	—	—	—
	11th ,, ..	**36**	**32**	28	—	—	—	—	—	—
	13th ,, ..	**33**	28	—	—	—	—	—	—	—
	15th ,, ..	29	—	—	—	—	—	—	—	—

U = Unadjusted.
C = Adjusted by girth.
E = Adjusted by previous crops.

[0] Adjustment not significant.
[1] Adjustment significant ($P<0{\cdot}05$).
[2] Adjustment highly significant ($P<0{\cdot}01$).
[3] Adjustment very highly significant ($P<0{\cdot}001$).

Incidentally, a correction upon girth is possible even when the trees have not been under observation since planting but this is not so for a correction upon previous crops.

It will be seen from Table II that adjustment upon girth works better for the longer periods and increases in effectiveness with lateness of application. Adjustment upon previous crops also is more effective when the trees are old but it is of dubious value for the longer experimental periods.

DISCUSSION.

The fact that the different trials were not equally uniform is hardly surprising, though it is not clear why they differed in the way they did. Trial I (Cox on I) was planted with carefully selected trees and it was hoped that it would give crops having a coefficient of variability of no more than 20 per cent. That it has not done so is probably due to the large blocks which have not effectively eliminated the high soil variability. Also, none of the trees grew really well whether with potash or without. It has been commonly supposed that the dominant sources of variation lie in the trees rather than in the soil, though there is evidence to the contrary (Brase and Tukey, 1936). The present results suggest that soil variation matters and should, therefore, be reduced as far as possible by the use of small blocks. Trial II (Cox on IX) was planted in the first place as a commercial field and this may well explain why it proved to be more variable than Trial III (Lane's on XII) which was always part of an experiment. The high variability for Trial IV (Worcester on Free) is doubtless the result of using non-clonal rootstocks planted as maidens. Selection of uniform rootstocks could hardly have been effective with the trees planted before vigour had had a chance to show itself.

These results also cast light on the old question—" How long does a plantation take to settle down in variability ? " Apparently, about ten years from budding, rather more for a vigorous rootstock, rather less for a dwarfing one. It is sometimes said that trees are variable until they have borne a good crop, but this is at best a partial truth. Thus, in Table II, Trials IA and IB (Cox on I), the crops in the two-year period after the 13th year gave a high coefficient of variability although the trees had already had some large crops and should have " settled down ". The broad principles do indeed appear to be that (*a*) large crops are less variable than small and (*b*) crops in initial periods are variable until the trees have given at least one large crop. Very many exceptions occur. The general principles are easily cut across by the incidence of disease and by differential fruit drop, while trees may be biennial in different degrees and not even biennially in phase with one another. It thus seems possible that unequal shelter may be a potent source of these occasional high variabilities.

For trials that begin with mature trees it will be noted that variability is often still falling after six years, so an experiment should not be abandoned after a shorter period solely on the grounds of the non-significance of the results. Of course, if the treatment is of the sort that is applied once and is likely to have a smaller effect in each successive year, the position is different.

At its best, the adjustment recommended will reduce variability by more than one-quarter. Although it is not always as effective as this, it is safe to say that, on the whole, its use enables two replicates to do the work of three where adjustments are not made. It must be emphasized that it is most effective for long experimental periods with trees that are no longer young. In other circumstances it may give disappointing results.

In the past it has been commonly supposed that like is best corrected by like, i.e. trunk thickening by previous trunk thickening, extension growth by previous growth, crops by previous crops and so on. For example, Parker (1942) working with citrus, found that adjustment of crop was carried out better by the previous cropping records than by trunk area of cross-section. On the other hand, Lutz (1938) found that size of pecan trees at planting gave little indication of future growth but that it did indicate future cropping. With apples, it appears from the results presented here that girth is more closely related to cropping than are earlier crops, thus providing another exception to the rule.

That trunk measurements of apple trees may be used in this way is shown by the work of Sudds and Anthony (1928) and of Yeager and Latimer (1939), though the latter apparently preferred previous crops. Wilcox (1940-41) thought that the best adjustment using trunk measurements could be made not from the trunk girth itself (Adjustment C) or the area of the cross-section (Adjustment D) but from an intermediate value (girth $^{3/2}$). He is almost certainly right but it is unlikely that the advantages of such a correction will repay the extra computing involved.

The failure of the method of Papadakis (Adjustment A) is confirmed by several unavailing attempts that have been made at East Malling in recent years to reduce variability in this way. This failure implies that local soil variation does not usually affect an area much larger than that occupied by a single tree. Conversations with workers from overseas have suggested to the writer that this adjustment is of greatest use where many native smallholdings have been thrown into one to form an experimental field and that it is often ineffective where the whole area of the trial has been farmed in one piece for a long time. The failure of the nursery records (B and C) to forecast later behaviour is possibly due to the custom in England of applying severe root pruning at the time of planting followed by quite drastic methods of tree-forming, a practice that must largely obliterate the nursery variation.

SUMMARY.

The variability in cropping of four series of apple trees was examined to see how it was affected by age of tree and length of experimental period. Coefficients of variability fell off with increasing length of experimental period and tended to decrease with increasing age of tree. Trees appear to settle down in variability after about ten years from budding. Crop figures can usefully be adjusted using a covariance correction upon trunk girth at the beginning of the experimental period, but not if the treatments are applied right from the time of planting. In this case, no useful means of adjusting the figures were found.

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EFFECTS OF THE ACCUMULATION OF VOLATILE SUBSTANCES PRODUCED BY APPLES IN GAS STORAGE

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In a previous communication (1947) some of the effects of temperature and gas mixtures ($5\% O_2 + 95\% N_2$ and $5\% O_2 + 10\% CO_2 + 85\% N_2$) on the ethylene and "odorous" fractions of the volatile substances produced by apples were shown. In these experiments, using synthetic gas mixtures, King Edward apples were stored in large desiccators in which the CO_2 of respiration and the volatile substances were not allowed to accumulate because the rate of flow of the gas mixture was sufficient to prevent this occurring. It was then found that if the CO_2 in the storage atmosphere was increased to 10% and the O_2 reduced to 5%, the rate of production of both volatile fractions at 15° C. was considerably below that in air.

These conditions are not found in commercial gas stores, where the desired concentrations of CO_2 and O_2 are obtained from the respiratory activity of the fruit and are then maintained by controlled atmospheric ventilation and the use of scrubbers when necessary to absorb surplus CO_2 (Hardy, 1937). At the same time an accumulation of volatile substances takes place in the store.

Experiments were therefore undertaken to compare the volatile production by apples when they are stored under commercial gas storage conditions and when they are stored, as in our previous experiments, without allowing the volatile substances to accumulate. For this comparison the apples were stored at 5° C. in (1), metal boxes in which the products of respiration were allowed to accumulate until the concentrations of CO_2 and O_2 were approximately 8% and 13% respectively and were then maintained to within 1% of this composition by means of a slow stream of air; (2), a desiccator through which flowed a synthetic mixture of $8\% CO_2 + 13\% O_2 + 79\% N_2$ at a rate sufficient to prevent the CO_2, due to respiratory activity, rising to more than 0·1–0·3%; (3), a desiccator in which the respiration rate in air at 5° C. of a sample of King Edward apples was also recorded at the same time.

EXPERIMENTAL.

King Edward apples were obtained from the same grower as were those in the previous experiments. The fruit was picked on 23rd October, 1947 and arrived at the laboratory on the following day. The apples were in good condition; the majority were hard and green but a few were changing colour to yellow or red. Those with injury marks were rejected and the remainder were sampled and weighed.

(1) Two oblong air-tight metal boxes or cabinets each of 144·5 litres capacity were fitted with a tap at each end of the lid, a tube from one tap extending to the bottom of the cabinet, so that the air drawn in at one end and sucked out at the other would pass through the entire length of the cabinet.

Each cabinet held 63·5 kg. of apples which were placed on a wooden rack to prevent direct contact with any moisture condensing on the bottom. One tap on each cabinet was connected with the air supply pipe from outside the building, and the other to a check gas sampling tube fitted with a by-pass to prevent interruption of the flow when gas analyses were made. The sampling tube of each cabinet was joined to a length of capillary tubing and these were finally united by means of a T piece with a flow-meter, which had been calibrated in this position at 5° C. The combined stream of gas flowed through a master gas sampling tube with a by-pass, to two Dreschel bottles in series, with sintered spoons, containing concentrated H_2SO_4 and activated H_2SO_4* for absorption of the odorous and ethylene fractions of the volatile substances respectively. The bottles were connected with the suction line, fine adjustment being obtained with needle valves and a mercury bottle. The cabinets were sealed and left for 24 hours before ventilation was started, at a rate of 1·8 litres per hour.

At the end of two days the CO_2 content of the cabinets was found to be 8% and the rate of flow was then adjusted to keep it within 1% of this level; the rate varied from 3 to 5 litres per hour throughout the storage period.

The composition of the combined stream was determined daily and occasional analyses of the gas from each cabinet were also made. Duplicate determinations of the carbon content of the H_2SO_4 from the Dreschel bottles were made at weekly intervals by the wet combustion method of Raistrick (1931).

(2) A desiccator of 13 litres capacity was used which held 4 kg. of apples. A gas mixture containing 8% CO_2+13% O_2+79% N_2 was released from a high pressure cylinder fitted with a needle valve and was passed through a flow-meter calibrated in this position at 5° C. It was then humidified by bubbling through dilute H_2SO_4 and flowed through the desiccator into a gas sampling tube fitted with a by-pass and then into two Dreschel bottles containing concentrated H_2SO_4 and activated H_2SO_4 respectively. A rate of approximately 2·5 litres an hour was sufficient to prevent accumulation of CO_2 in the desiccator. Analysis of the gas stream was made at frequent intervals, and a determination of the carbon content of the sulphuric acids was made weekly. Determinations of the gas mixture in the cylinders were also made.

(3) A sample of 3 kg. of apples was used for the measurement of the CO_2 produced when the fruit was stored in air in a desiccator of 13 litres capacity.

A stream of air from outside the building was passed over soda-lime to remove CO_2 and was humidified by bubbling through 8% KOH. After passing through the desiccator, the CO_2 of respiration was absorbed in N.NaOH in Pettenkofer tubes and estimated daily by titration with N. HCl after addition of $BaCl_2$ solution. These conditions maintained 0·5% approximately of CO_2 in air round the fruit.

RESULTS.

In conformity with the experiments in 1947, the rate of production of the volatile substances is expressed as ml. of CO_2 per 10 kg. per hour, and is shown in Figs. 1 and 2.

The true concentration of the "odorous" volatiles in the gaseous stream cannot be estimated as the constituents of this fraction are not known with any

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* 2% w/v Ag_2SO_4 in concentrated H_2SO_4.

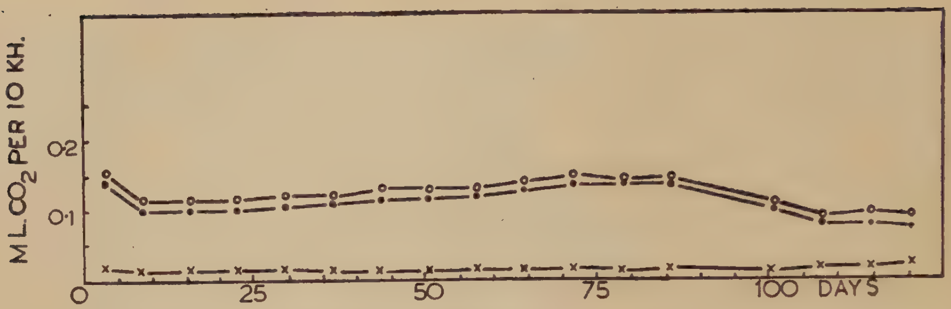

FIG. 1.

Production of volatile substances by King Edward apples stored at 5° C. in cabinets in which these substances are allowed to accumulate.

· —— · Ethylene fraction
× —— × Odorous fraction
o —— o Ethylene and Odorous fractions
} Expressed as ml. CO_2 per 10 Kg. per hour.

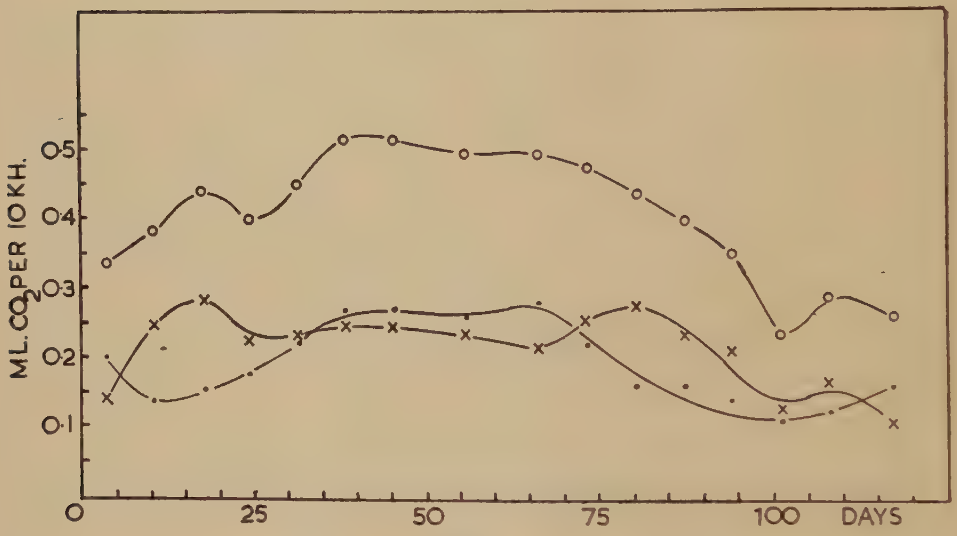

FIG. 2.

Production of volatile substances by King Edward apples stored at 5° C. in a desiccator in 8% CO_2, 13% O_2 and 79% N_2 without accumulation of these substances.

· —— · Ethylene fraction
× —— × Odorous fraction
o —— o Ethylene and Odorous fractions
} Expressed as ml. CO_2 per 10 Kg. per hour.

degree of accuracy and therefore the concentration of both fractions is shown in Figs. 3 and 4 as mg. of carbon per 100 litres of gas.

In the cabinets (Expt. I), the rate of production of CO_2 by respiration is calculated from the flow-meter readings and the results of analysis of the gas samples. In the desiccator (Expt. II) this rate is calculated in a similar manner, allowance

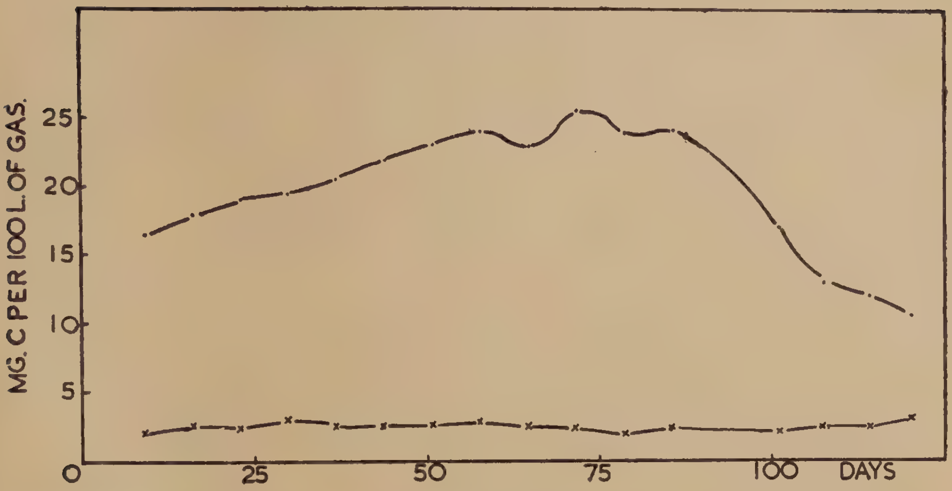

FIG. 3.

Concentration of volatile substances in the atmosphere in the cabinets in which these substances are allowed to accumulate.

· —— · Ethylene fraction } Expressed as mg. C. per 100l. of gas.
× —— × Odorous fraction

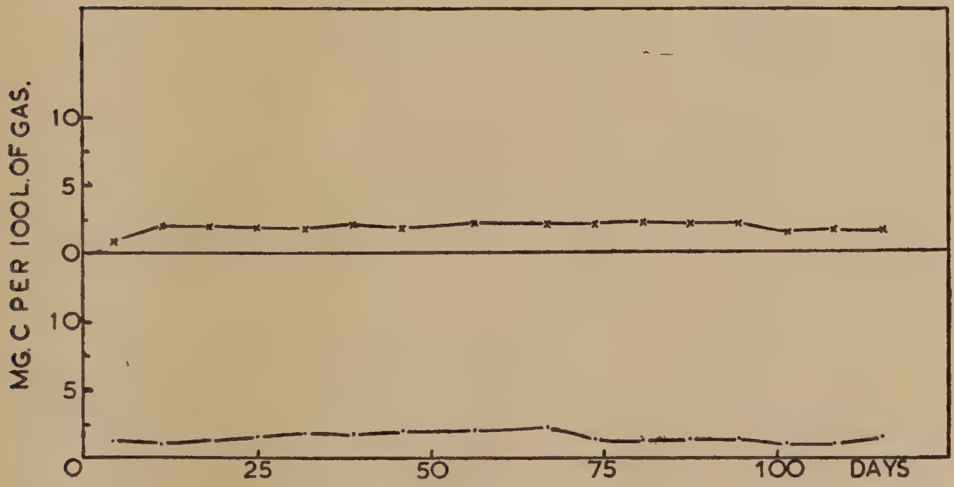

FIG. 4.

Concentration of volatile substances in the atmosphere in the desiccator in which these substances are not allowed to accumulate.

· —— · Ethylene fraction } Expressed as mg. C. per 100l. of gas.
× —— × Odorous fraction

being made for the CO_2 present in the synthetic gas mixture. In both cases the rate is expressed as ml. of CO_2 per 10 kg. per hour and is shown in Fig. 5, together with the rate of CO_2 production in air (Expt. III).

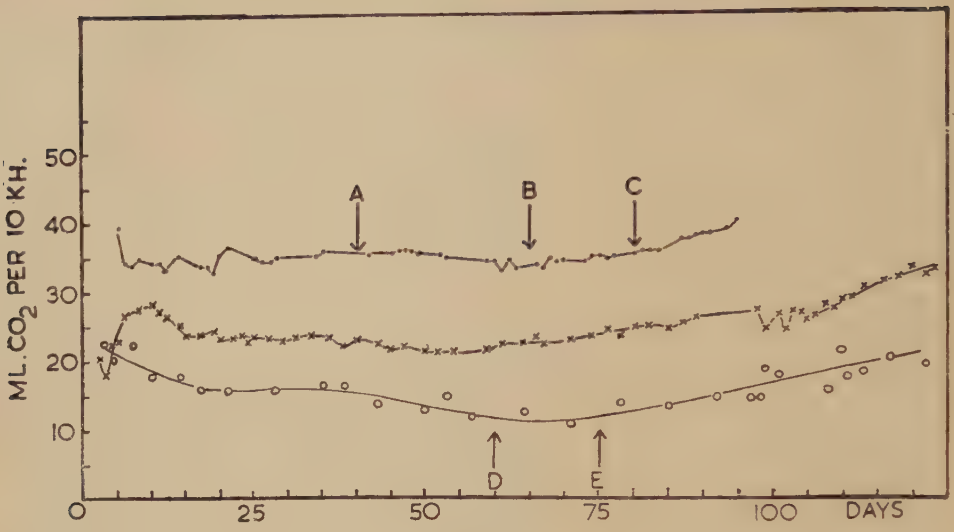

FIG. 5.

Production of CO_2 by King Edward apples stored at 5° C.

• —— • In air in desiccator. A = Colour change and slight scald. B = One rot. C = Two more rots.

× —— × In cabinets with accumulation of volatile substances and CO_2 until concentration of $CO_2 = 8\%$.

o —— o In synthetic mixture of $8\% \ CO_2 + 13\% \ O_2 + 79\% \ N_2$ in desiccator. D = Colour change or slight scald. E = All with slight scald, one severe and one rot.

In Table I, the ratios of the ethylene to the odorous fraction are tabulated and are calculated at the mean of each period for which carbon was determined.

At the end of the experiment (after 120 days at 5° C.) an examination of the condition of the fruit in the cabinets and in the desiccator was made (Table II).

DISCUSSION.

A comparison of the rates of production of volatile substances is shown in Figs. 1 and 2 from which it is evident that an accumulation of volatiles in the storage atmosphere lowers their production rate.

The production of volatile substances by the apples in the desiccator is approximately three times that in the cabinets. Thus, in the desiccator it rises from 0·32 to 0·52 ml. CO_2 per 10 kg. per hour during a period of 45 days, after which it falls to 0·25 ml. during the next 60 days whereas, in the cabinets, after a short initial fall, it rises gradually from 0·11 to 0·15 ml. in 70 days, with a subsequent fall to 0·09 ml. in the ensuing 35 days.

The main feature as regards the rate of change of production of total volatiles is a 50% rise to a peak which occurs much later with the fruit stored in the presence of an accumulation of its own volatiles than with the fruit stored under conditions which provide for a continuous removal of volatiles.

TABLE I.

Ratio of ethylene to odorous constituents in the volatile products from Apples stored at 5° C. in 8% CO_2 + 13% O_2 + 79% N_2.

With accumulation of volatile substances.		Without accumulation of volatile substances.	
Days.	Ratio.	Days.	Ratio.
2	10·0 : 1	3½	1·4 : 1
7½	8·0 : 1	10½	0·55 : 1
14½	7·1 : 1	17½	0·54 : 1
21½	7·8 : 1	24½	0·60 : 1
28½	7·1 : 1	31½	0·95 : 1
35½	8·6 : 1	38½	1·20 : 1
42½	9·2 : 1	45½	1·10 : 1
49½	8·8 : 1	56	1·05 : 1
56½	9·1 : 1	66½	1·30 : 1
63½	8·9 : 1	73½	0·85 : 1
70½	10·5 : 1	80½	0·59 : 1
77½	12·3 : 1	87½	0·69 : 1
84½	9·5 : 1	94½	0·68 : 1
95½	7·8 : 1	101½	0·86 : 1
106½	5·1 : 1	108½	0·76 : 1
113½	4·7 : 1	117½	1·50 : 1
119½	3·5 : 1		

The volatile substances produced by the apples in air were not determined, but if the rate of production in 8% CO_2 + 13% O_2 + 79% N_2 is compared with that obtained in air at the same temperature (5° C.) in 1947 experiments, a decrease in the rate similar to that at 15° C. is indicated although the apples are not at the same stage of maturity.

When the ethylene and odorous fractions are considered separately, it appears that the rise to a peak and subsequent fall of total volatiles is due mainly to the ethylene fraction. It is also evident that the effect of volatile accumulation in depressing the rate of production of volatiles is much more pronounced on the "odorous" than on the ethylene fraction.

There is a greater variation in the rate of production of the two fractions in the desiccator than in the cabinets, a fact which cannot be attributed to any difference in the method of measurement and must therefore be regarded as genuine. It is tentatively suggested that the accumulation of volatiles not only depresses their rate of production but also lessens the possible effect of small changes in conditions.

The concentration of the two fractions is seen in Figs. 3 and 4 which show that there is comparatively little accumulation of volatiles in the desiccator, the level being from 1 to 2 mg. of carbon per 100 litres of gas for both fractions whereas, in the cabinets, the concentration of ethylene is high at the start and rises from 16 to 25 mg. of carbon per 100 litres in approximately 70 days and then falls sharply to 10 mg. in the last 48 days. The concentration of the "odorous" fraction is only slightly higher in the cabinets than in the desiccator and keeps between 1·9 and 3 mg. carbon per 100 litres throughout.

The ratios of the ethylene fraction to the odorous fraction in the two experiments show a marked difference. In the cabinets this ratio is much higher, the mean value being approximately 9 : 1 for the first 100 days, after which it falls to 4·4 : 1 for the last 20 days. In the desiccator the ratio only rises slightly above 1 : 1 during the middle period of storage, falling below this value at the beginning and end of storage. This difference in the ratios is mainly due to the greater depressing effect which the accumulation of volatile substances exerts on the odorous fraction. The high ratio observed in the cabinets is in line with the high ratios observed in commercial gas stores.

The rate of CO_2 production of the apples in air can be seen in Fig. 5 together with that of the fruit stored in the cabinets and in the desiccator. The depressing effect of the presence of 8% CO_2 on the respiratory activity is evident in both cases of gas storage, but is greater in the desiccator, that is when the volatiles are not allowed to accumulate.

The depressing effect of CO_2 on respiration of apples at 10° C. has been studied by Kidd and West at all stages of storage life. They found (1933) that with fully grown Sturmer Pippins there was a marked depression of the respiratory activity in the post-climacteric phase, but no marked effect in the pre-climacteric. With Bramley Seedlings (1937), CO_2 produced an immediate but transitory increase in activity in the case of very immature fruit. This was followed by a continuous fall in activity below that in air. With more mature but pre-climacteric fruit, the depression, after the initial stimulation, was more gradual and the rate of production of CO_2 did not fall below that in air until the onset of the climacteric in air. Climacteric rises were not produced by mature or immature Bramleys when stored in CO_2. Kidd and West (1937) also found that when immature pre-climacteric Bramleys were stored in 10% CO_2, 20% O_2 at 10° C., the rate of CO_2 production could be stimulated with ethylene.

In the present experiment, it is doubtful whether the apples were pre- or post-climacteric at the time of picking, although the record of respiratory activity in air suggests that the climacteric was well advanced. It seems to be probable that where the volatiles accumulate, as in the cabinets, the concentration of ethylene which rises to 16·5 mg. C. per 100 litres (150 parts per million) within the first few days causes the rise in the rate of CO_2 production which is maintained throughout. In all three instances, the upward drifts in the curves of CO_2 production towards the end of the storage life are attributable to the occurrence of rots.

Table II sets out the condition of the apples in the cabinets and desiccator at the end of the experiment. The apples in the cabinets were not visible during storage, but their final condition showed that scald and rots were more pronounced in the cabinets than in the desiccator. The atmospheric conditions in the cabinets, judged by the appearance of the fruit, must have been more humid than those in the desiccator although there was no collection of moisture on the floor. In both containers the apples were green (where not scalded), crisp and juicy, as judged by eye and palate, with no marked distinction between the two sets but no critical tests were carried out.

Whether these results throw any light on the cause of apple scald can now be discussed. Clearly, the conditions in the cabinet which allow the volatile substances to accumulate result in more scald than in the desiccator, where these

TABLE II.

Condition of Apples after storage at 5° C. in $8\%\ CO_2$, $13\%\ O_2$, $79\%\ N_2$.

Condition.	In the cabinets, with accumulation of volatile substances.	In the desiccator, without accumulation of volatile substances.
	% of total apples.	% of total apples.
Fair. Very slight scald	5	25
Slight scald (less than ¼ of surface)	7	45
Medium scald (less than ½ of surface)	20·5	25
Severe scald (more than ½ of surface)	14·0	—
Scald and rots	47·5	5
Complete rots	6	—

substances are being continuously removed. The concentrations of these volatile substances in the storage atmosphere are known, but those in the apple must be greater by amounts proportional to their relative rates of production, so that the internal concentration of ethylene in the apples is probably much greater in the cabinets than in the desiccator, whilst in the case of the odorous fraction the reverse may even be true.

That ethylene causes scald directly seems unlikely, as it is known that when oiled paper is used for the prevention of apple scald, it must be in close proximity to the apple, which indicates that it absorbs a compound of higher molecular weight than that of ethylene. If, on the other hand, the odorous compounds are considered, then the virtual suppression of the formation of the odorous fraction in the cabinets is a very striking fact, as is the relatively very large increase in its rate of production which is associated with a very small decrease in its concentration in the storage atmosphere.

We therefore suggest that the causative conditions for scald may be the accumulation of precursors of the odorous volatiles.

SUMMARY.

1. The accumulation of volatile substances in the storage atmosphere containing $8\%\ CO_2 + 13\%\ O_2 + 79\%\ N_2$ decreases their rate of production, the effect being more pronounced on the odorous than on the ethylene fraction.

2. The ratio of the ethylene to the odorous fraction is considerably greater when the volatile substances are allowed to accumulate in the store than when there is no accumulation.

3. A high concentration of volatile substances in the storage atmosphere coincides with a marked increase in the amount of scald on the sample of King Edward apples used.

4. In the present experiments, the respiratory activity of the fruit in $8\%\ CO_2 + 13\%\ O_2 + 79\%\ N_2$ with and without accumulation of volatile substances, is lower than in air. The depressing effect is greater when the concentration of

volatile substances is low, but the general drift of the respiration rate is similar in both cases.

The work described above was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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SOME OBSERVATIONS ON THE CHEMICAL CHANGES EFFECTED BY THE STEAM STERILIZATION OF GLASSHOUSE SOILS

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The steam sterilization of glasshouse soils has now assumed a position of great practical importance and most progressive growers steam their soils regularly. While considerable advances have been made in the technique and methods of steam sterilization, there appear to have been very few scientific investigations into the nature of the profound chemical and biological changes effected by steaming. It has been known for some time (1), (2), (3), and (5), that the partial sterilization of soil leads to the accumulation of ammonia and most growers are aware of the soft, rank growth likely to follow steaming.

In some instances, particularly in the preparation of seed and potting composts, steam sterilization causes a severe check in growth. This aspect has been studied within the last ten years (2), and as a result it has been possible to recommend the practical steps to be taken to produce sterilized composts nearly always giving optimum results. Nevertheless quantitative estimations of the chemical changes brought about by steaming have not been largely undertaken, and this paper describes an attempt to measure some of these changes.

The primary object of steaming is, of course, the destruction of pathogenic organisms but the soil is also affected physically, chemically and biologically. We have not concerned ourselves with the physical changes, although often these are quite obvious. Unfortunately, it has been impossible to examine the fluctuation in the population of soil micro-organisms and we have been confined to measuring the ammonia produced as a result of these biological changes. Steaming causes direct chemical changes in the soil apart from those brought about by the effect on micro-organisms. There is, for instance, an increase in the water-soluble organic matter, and often an increase in the water-soluble and exchangeable manganese. Under some conditions ammonia, apart from that produced by micro-organisms, probably results from the direct action of the steam on the soil.

It is clear that the consequences of steaming a soil are very complex and demand intense and involved investigation by chemists, biologists and plant physiologists if much progress is to be made in our knowledge of the subject. These preliminary investigations only take account of some of the simpler chemical changes brought about when a soil is steamed. It is hoped to consider, at a later date, the relation between these changes and the effect on plant growth.

EXPERIMENTAL.

(1) Experiments in Commercial Glasshouses.

With the co-operation of a few growers, samples of soil were taken before steaming and at intervals afterwards. The method of steaming in all cases was the

" Hoddesdon " pipe system but no two growers seem to steam in exactly the same way. Sometimes the pipes are laid at 20 inches, sometimes at 18 inches and even at 12 inches where steaming has been previously carried out. The time of steaming also varies slightly but, in general, steam is passed for 15 to 20 minutes ; most soils seem to reach a temperature of 212° F. in about 15 minutes and steam is passed for a further 5 to 10 minutes. To reduce the sampling errors, the first soil sample was taken from the top 12 inches after the pipes had been placed in position, the area covered being about 5 square yards. Subsequent soil samples were taken in the same way from this relatively small area. About 5 lb. of soil were taken for each sample, sieved through a quarter-inch sieve and analysed the same day.

Three centres were chosen for the steaming and the soils and analyses were as follows :

Centre 1.—An old cucumber soil steamed for the first time, organic medium loam.

Centre 2.—A rather stiff loam, steam sterilized every third year. Heavily manured and fertilized for many years.

Centre 3.—A relatively poor light sand, low in organic matter and steamed for the first time.

ANALYTICAL DATA.

	Centre 1.	*Centre 2.*	*Centre 3.*
% Loss on Ignition	20·3	11·4	9·0
% Total Nitrogen	0·61	0·42	0·28
pH	5·48	6·70	5·50

Centre 1.

There was no manurial treatment and the soil was left undisturbed after steaming, until it was flooded and tomatoes were planted on 3rd March (Table I).

There was no immediate large increase in the ammonia concentration, but a gradual one, after a large jump in the first week, over a period of a month or so. The nitrate concentration was high initially and the fluctuating concentration may

TABLE I.

Centre 1. Analyses of soil. Results expressed on the oven-dry soil basis.

Date and Treatment.	p.p.m. Ammonia Nitrogen.	p.p.m. Nitrate Nitrogen.	p.p.m. Total Inorganic Nitrogen.	p.p.m. Water-Sol. Mn.	pH.	Moisture.
Before steaming :						
11.1.45	14	140	154	nil.	5·48	26·8
After steaming :	16	154	170	2·9	5·69	40·3
18.1.45	36	172	208	4·2	5·43	36·6
26.1.45	39	137	176	3·5	5·63	34·7
9.2.45	47	156	203	2·6	5·52	32·1
16.2.45	52	145	197	1·7	5·98	34·7
After flooding and planting	50	92	142	1·6	6·08	32·2

be a measure of soil variations even over such a small sampling area. The nitrate fell markedly after flooding and planting. Steaming increased the total inorganic nitrogen from about 155 to 200 p.p.m., but the original proportion of ammonia nitrogen to nitrate nitrogen changed from 1 : 10 to 1 : 3. Water-soluble manganese increased slightly and then gradually decreased. There was a tendency for the pH to rise, particularly as the nitrates fell. The moisture content rose considerably more than is usual on steaming, the normal increase being 1 to 2 per cent.

The tomato crop was heavy, although it received no more nitrogen in the base or top-dressing.

Centre 2.

At this centre a small area was left untreated after steaming and, except for watering, received nothing else throughout the season (treatment A). The rest of the house was dressed with 15 tons F.Y.M. per acre after steaming, 4 oz. per sq. yard $CaCO_3$, and given the usual top-dressings (treatment B). Winter lettuce were removed just prior to steaming the soil and tomatoes were planted on 20th March (Table II).

TABLE II.

Centre 2. Analyses of soil.

Date.	Treatment.	p.p.m. Ammonia Nitrogen.	p.p.m. Nitrate Nitrogen.	p.p.m. Total Inorganic Nitrogen.	pH.	Moisture.
8.3.45	Before steaming ..	12	65	77	6·71	28·3
	After steaming ..	13	52	65	6·69	29·1
16.3.45	A. flooded ..	47	34	81	6·86	28·9
	B. ,, ..	89	31	120	7·20	36·0
26.3.45	A.	86	55	141	6·64	27·0
	B.	101	71	172	6·88	29·3
6.4.45	A.	60	82	142	6·81	30·2
	B.	42	115	157	6·71	31·6
1.5.45	A.	15	41	56	6·75	27·6
	B.	25	178	203	6·53	28·1

As at the first centre, the ammonia concentration increased rapidly during the first week, particularly on the dung-treated plot ; a further appreciable increase was noted after another ten days but thereafter, owing to nitrification, the ammonia concentration fell and the nitrate concentration increased. Nitrification had obviously started 18 days after steaming. Six weeks after planting the total inorganic nitrogen was much higher with treatment B than with treatment A. The original ammonia nitrate ratio was about 1 : 5, but when the tomatoes were planted the ammonia concentration was higher than the nitrate. Only traces of water-soluble manganese were found in any of the samples. The pH of the untreated soil remained fairly constant but the pH of the soil limed after steaming rose initially and fell rapidly on the formation of nitrate. The crop was heavy and only slightly lower on the section untreated after steaming compared with the heavily manured area but the plants were definitely less vigorous.

Centre 3.

This soil was not dunged or treated with fertilizers during the time samples were taken but one area was not flooded (treatment A, Table III) and the other was (treatment B).

TABLE III.

Centre 3. Analyses of soil.

Date.	Treatment.	p.p.m. Ammonia Nitrogen.	p.p.m. Nitrate Nitrogen.	p.p.m. Total Inorganic Nitrogen.	p.p.m. Water-Sol. Mn.	pH.	Moisture.
26.2.45	Before steaming	14	63	77	Trace	5·64	19·2
	After steaming	16	66	82	3·1	5·36	19·2
5.3.45	A. not flooded	24	53	77	2·1	5·57	18·3
	B. flooded	13	17	30	Trace	5·94	23·1
23.3.45	A. not flooded	23	50	73	2·0	5·34	16·6
	B. flooded	26	39	65	Trace	5·63	17·4

It is obvious that this soil was poorer than the others and the increase in ammonia was considerably less than in the richer soils. Some of the ammonia and much of the nitrate was leached from the top 12 inches on flooding but within a fortnight the concentrations were approaching those on the unflooded area. There was a slight increase in water-soluble manganese on steaming, all of which was removed by flooding.

This house (Cold Dutch) was planted with tomatoes and one half was left without any further nitrogenous treatment while the other half received the normal base and top-dressings. The plants were definitely paler in the untreated part but they cropped just as heavily, at nearly 40 tons per acre.

(2) LABORATORY EXPERIMENTS.

In the course of this work in commercial houses it became increasingly obvious that experiments would have to be carried out under controlled conditions in the laboratory if accurate information of a really fundamental nature were to be obtained. It was realized that the investigation would probably lose some of its practical value as it is extremely difficult to reproduce in a laboratory the biotic conditions prevailing in a glasshouse.

For the laboratory steaming, a small wooden box was devised, capable of holding about 10 lb. of soil. The box was 12 inches long, 6 inches wide and 8 inches deep. Through a hole at one end, near the bottom of the box, was inserted a metal tube pierced with holes and plugged at one end. Steam was blown in from the laboratory supply, using a large flask as a trap to collect condensed steam. During steaming, the box was covered with asbestos board through which a thermometer was inserted into the soil. Only ammonia and nitrate were determined and it was first necessary to compare the laboratory steaming with commercial steaming, in order to see if there were any marked differences in the results. For this purpose another centre was chosen, the soil being of a dark sandy nature, and having been used for tomatoes and lettuce under glass for many years. It had been regularly steamed and had the following analysis:—

pH	6·52
Loss on ignition	12·2%
Total nitrogen	0·41%

The effects of the following treatments on the concentration of ammonia and nitrate were observed:

Treatment A.

Soil was taken from a small area before steaming and was kept as a control in a glass stoppered bottle in the laboratory; put through a ¼ inch sieve.

Treatment B.

Was the same as A, but the soil was steamed in the laboratory for 15 minutes until a temperature of 212° F. was reached and then for a further 5 minutes; it was bottled immediately after steaming.

Treatment C.

The sample was taken from the glasshouse immediately after steaming; it was sieved and bottled and was kept in the laboratory.

Treatment D.

Samples were taken at intervals from the steamed soil in the glasshouse and were sampled and analysed on the same days as the samples kept in the laboratory. Although this small area was left uncropped it was occasionally watered with the rest of the house. The results are shown in Table IV and are expressed as p.p.m., on the oven-dry soil basis.

TABLE IV.

Determinations of ammonia N and nitrate N in soil under treatments A, B, C, D.

Treatment	A.		B.		C.		D.	
Date.	p.p.m. NH_3–N.	p.p.m. NO_3–N.	p.p.m. NH_3–N.	p.p.m. NO_3–N.	p.p.m. NH_3–N.	p.p.m. NO_3–N.	p.p.m. NH_3–N.	p.p.m. NO_3–N.
5.3.46 ..	12	82	12	77	14	61	14	61
12.3.46 ..	7	88	25	76	27	67	55	47
19.3.46 ..	7	90	32	73	34	64	62	32
26.3.46 ..	10	92	42	79	38	73	74	—
9.4.46 ..	10	97	44	76	10	110	27	55
24.4.46 ..	8	95	43	78	6	110	10	61
8.5.46 ..	7	97	18	104	7	110	2	28

The unsteamed soil behaved quite normally, ammonia remaining fairly constant and the nitrate slowly increasing. The ammonia concentrations in the treatments B and C agreed quite closely for three weeks after steaming and behaved in a similar way to normal glasshouse steaming (see Table I). While the nitrate concentration in treatment B remained almost constant for two months, it increased sharply in treatment C after five weeks and this was accompanied by a similar decrease in the ammonia concentration.

Nitrification had obviously started in treatment C after about three weeks. This may mean that the glasshouse steaming was not as efficient as the laboratory steaming in that all the nitrifying bacteria were not killed. It is possible, however, that the soil had become re-infected when the bottle was opened for periodic sampling and analysis. This is unlikely in view of the fact that during the whole course of this investigation no other soil showed marked signs of nitrification so soon after steaming and bottling. Nitrification started in treatment B after about two months and the total ammonia and nitrate concentrations were very similar in treatments B and C. On coming to treatment D it is seen that far more ammonia (30 p.p.m. ammonia nitrogen) was produced in the first week than in treatments B and C but for the next two weeks the increases were similar. After the third week there was a big drop in the ammonia concentration and presumably nitrification, as in C, was proceeding rapidly. The nitrate concentrations in treatment D were very variable and must have been influenced by the watering the soil received or by other factors; they serve to indicate the errors likely to be experienced in investigations of commercial steaming. It seems clear from these results that the laboratory steaming gave substantially the same results as the glasshouse steaming in the soils stored under similar conditions in the laboratory (apart from the difference of renewed nitrification in the one instance) but ammonification proceeded to a greater extent in the soil steamed and left in situ under glass, at least for the first week. The most likely explanation of this difference is the retention of a higher temperature by the soil in a glasshouse for some days after steaming. On bottling steamed soil at 100° C. it is down to laboratory temperature in an hour or so, whereas a steamed glasshouse soil takes much longer to cool down. To check this point, a soil was steamed in the laboratory and a portion of this was bottled and allowed to cool rapidly in the normal way; another portion was bottled and placed in the oven at 100° C. and the temperature was gradually reduced for 24 hours to that of the laboratory. The results of the ammonia nitrogen determinations are shown in Table V.

TABLE V.

The effects on ammonia N production in soils, brought about by rapid and by slow cooling after steaming. Data in p.p.m. ammonia nitrogen.

	Soil steamed and cooled rapidly.	Soil steamed and cooled slowly.
After 1 day	21·7	39·0
After 8 days	31·6	55·2

These data lead one to suppose that there will be more ammonia formed in commercial steaming than in the laboratory steaming because of the maintenance of higher temperatures after steaming, and there will also be an earlier resumption of nitrification especially if the soil is accorded the usual treatment of watering, planting out, etc. As long as these differences are borne in mind one can carry out laboratory steaming in order to compare the effects of steaming soils of different moisture contents, at varying pH levels and for different lengths of time, and use these results to draw conclusions as to the probable effect of these factors in practice. These would be extremely difficult experiments to carry out in commercial glasshouses. It was decided therefore to use the more accurate laboratory steaming to investigate the effect of these various factors on the production of ammonia.

The production of ammonia in soils steamed for various times.

Two different greenhouse soils were used in this investigation and about ½ cwt. was sieved and each bulk was separately and thoroughly mixed. About 5 lb. of soil were taken for each of the different periods of steaming. The soil was brought to a temperature of 212° F. in 15 minutes and thereafter the different samples were steamed for 5, 10, 15, 30 and 60 minutes respectively, at 212° F. The soils were bottled immediately after steaming; they were stored in the laboratory and were analysed at regular intervals. The results are shown graphically in Fig. 1 (soil 1) and Fig. 2 (soil 2). The soils had the following analysis:

	Soil 1.	*Soil 2.*
pH	5·84	6·05
Loss on ignition	15·3%	19·2 %
Total nitrogen	0·46%	0·51%
Moisture	20·0 %	30·3 %

Soil 2 contained more fibre and undecomposed organic matter. It is seen from both graphs that the ammonia concentration increased for 7 to 8 weeks when it reached its peak. The total quantity produced after this time was not greatly different for the various times of steaming but in both samples slightly less ammonia was produced in the soil steamed for 60 minutes. In both instances more ammonia was produced after one day in the soil steamed for 60 minutes but for the following seven days ammonia production was considerably lower with these soils than with the others; thereafter the production of ammonia proceeded at approximately the same rate for all treatments. The most rapid production took place during the first four weeks. More than twice as much ammonia was produced in soil 2 as in soil 1, which is probably a reflection of the higher undecomposed organic matter content and the higher moisture content. The ammonia concentration in the steamed soil 1 was about twelve times greater than in the control after eight weeks, and in soil 2 about twenty times greater. The greater production of ammonia after one day in the soils steamed for 60 minutes is probably due to chemical breakdown, and the slower production of ammonia in the first week following is also interesting, indicating some factor at work slowing down ammonification. The nitrate concentration in soil 1 remained fairly constant for all treatments at 40 to 50 p.p.m. and for soil 2, at 115 to 125 p.p.m. NO_3-nitrogen. There was some evidence of a slight decrease after steaming and then of a slight increase during the last two weeks or so but these differences were not more than a few p.p.m. and may be due to sampling and analytical errors.

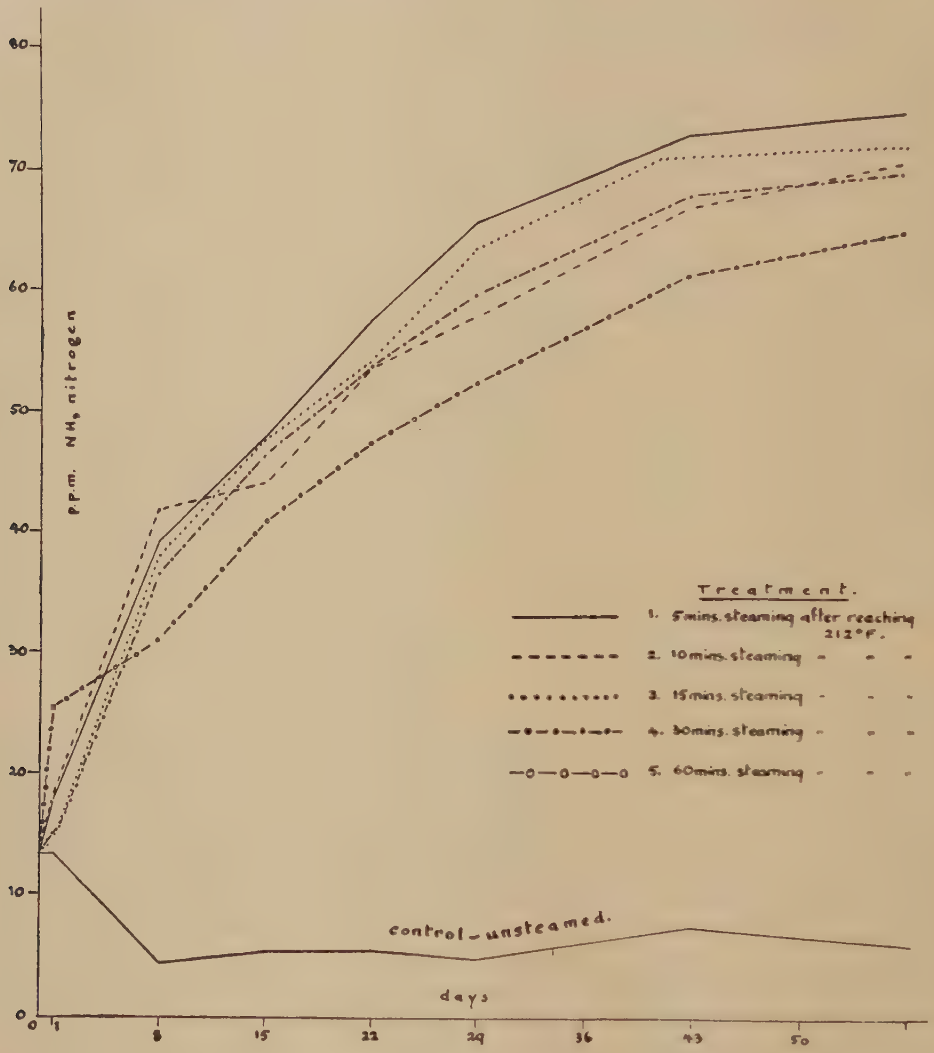

FIG. 1.
Production of ammonia in Soil 1, for various times of steaming.

It would appear from these results that ammonia production is not greatly different in soils steamed for 5 to 30 minutes at 212° F. Steaming for an hour probably effects some chemical breakdown of organic matter and immediate production of ammonia, and ammonia production by micro-organisms is thereafter retarded for a period of seven days or so. In the richer soil there was evidence that some chemical decomposition was brought about by steaming for 30 minutes but this was less pronounced than in the 60-minute steaming.

The production of ammonia in soils steamed at various moisture contents.

The first soil used in this experiment was soil No. 2 referred to above, a quantity of which had been allowed to dry out slowly in the laboratory over a period of 2 to 3 weeks until the moisture content was 16 to 17 per cent. One portion of soil was steamed without the addition of water, another with 26 to 27 per cent. water, and a third with 33 to 34 per cent. water. The time of steaming was 15 minutes to reach 212° F. and a further 5 minutes at 212° F. The steamed soils and control were kept in glass-stoppered bottles and were analysed at intervals (Table VI) for ammonia and nitrate contents.

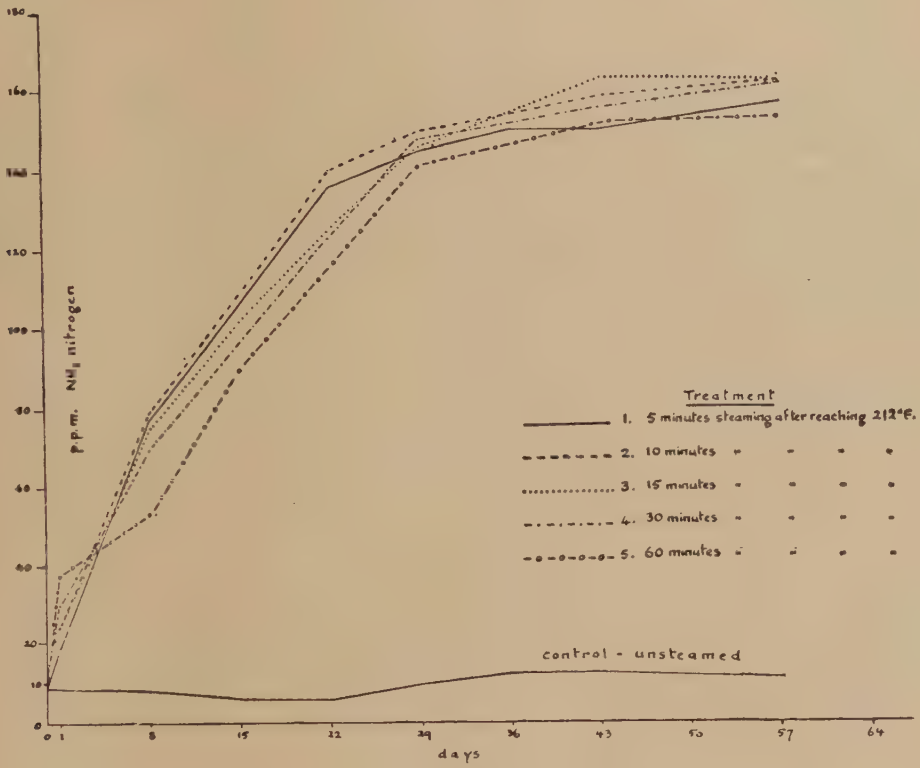

FIG. 2.

Production of ammonia in Soil 2 for various times of steaming.

It will be noticed that the ammonia content was very high in the unsteamed control, the slow process of drying out in the laboratory having increased the ammonia nitrogen from about 8 p.p.m. to over 60 p.p.m. The nitrate nitrogen content was very similar (approx. 130 p.p.m.) to the original nitrate content when the soil was

TABLE VI.

Showing p.p.m. ammonia nitrogen in samples of a soil (No. 2), steamed and unsteamed and having different moisture contents.

Date.	16–17% Moisture. Unsteamed.	18–19% Moisture. Steamed.	26–27% Moisture. Steamed.	33–34% Moisture. Steamed.
1.2.46	65	66	67	63
8.2.46	67	76	109	125
15.2.46	62	92	142	163
1.3.46	69	149	166	190

first brought into the laboratory. The production of ammonia in this soil merely on drying out was greater than that after steaming some soils and is an interesting point. There was a marked increase in production of ammonia at the higher moisture contents with this rich soil and the wetter the soil the more ammonia was produced. The nitrate contents remained fairly constant and there was no evidence of reduction at the highest moisture content. The experiment was repeated on a lighter soil with a lower moisture-holding capacity, at four different levels of moisture. The soil was extremely wet at 25 per cent. water. This soil was dried out rapidly in one day to avoid the accumulation of ammonia by prolonged drying and was then moistened up to the various levels. Conditions of steaming, etc. were similar to those of the previous experiment and the results are given in Table VII.

For this lighter soil, there was again clear evidence of a greater production of ammonia at the higher moisture contents but the amounts of ammonia produced were considerably less than in the previous soil. From the very high nitrate content it would seem that most of the readily decomposable nitrogen had already been converted into nitrate the concentration of which was nearly 250 p.p.m. nitrate nitrogen. The increase in ammonia produced seven weeks after steaming only amounted to about 30 p.p.m. The nitrate content remained fairly constant in the steamed soils except in the soil with the highest moisture where there was some indication of reduction.

On rich soils with much readily decomposable organic matter it seems likely

TABLE VII.

Showing p.p.m. ammonia nitrogen in samples of a light soil, steamed and unsteamed and having different moisture contents.

Date	9% Moisture. Unsteamed.	11% Moisture. Steamed.	17% Moisture. Steamed.	21% Moisture. Steamed.	25% Moisture. Steamed.
7.3.46	14	16	17	17	19
14.3.46	13	16	22	22	28
21.3.46	13	24	30	35	29
28.3.46	8	28	36	37	39
11.4.46	—	37	44	43	51
25.4.46	—	39	48	44	47

that steaming at high moisture contents will bring about the production of more ammonia than will steaming at lower moisture levels. On relatively poorer soils the effect is not so marked.

The production of ammonia on steamed soils of varying pH.

For this experiment a very acid field soil (soil 3) was chosen with the following analysis:

pH	4·3
Loss on ignition	7·85%
Total nitrogen	0·26%

A large bulk of soil was sieved through a ¼ inch sieve and one portion was bottled as control. Another portion was steamed in the ordinary way without liming, and others with 1 gram $Ca(OH)_2$/kilo (approx. 1 ton per acre $Ca(OH)_2$), 3 grams $Ca(OH)_2$/kilo and 5 grams $Ca(OH)_2$/kilo. The soils were bottled after steaming and the ammonia and nitrate contents estimated at intervals. The nitrate contents remained almost constant at 13 to 16 p.p.m. nitrate nitrogen and the ammonia contents are graphed in Fig. 3. It will be noted that for at least a

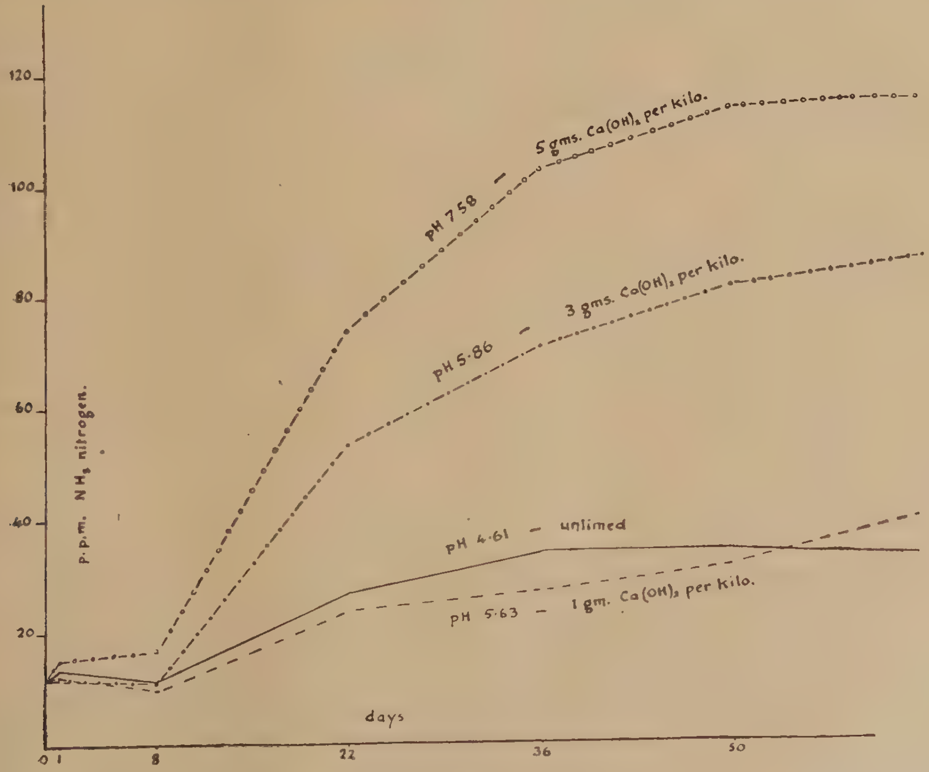

FIG. 3.

The effect of lime on ammonia production in steamed soils (Soil 3).

week there was little change in the ammonia content. After three weeks there was a very large increase of ammonia in the two most heavily limed soils. The soil which was limed at the lowest rate contained slightly less ammonia than the steamed unlimed soil and considerably less than the more heavily limed soils. A vigorous mould growth (*Pyronema glaucum*) was observed in the most heavily limed soil after three weeks. The ammonia content continued to increase for some weeks.

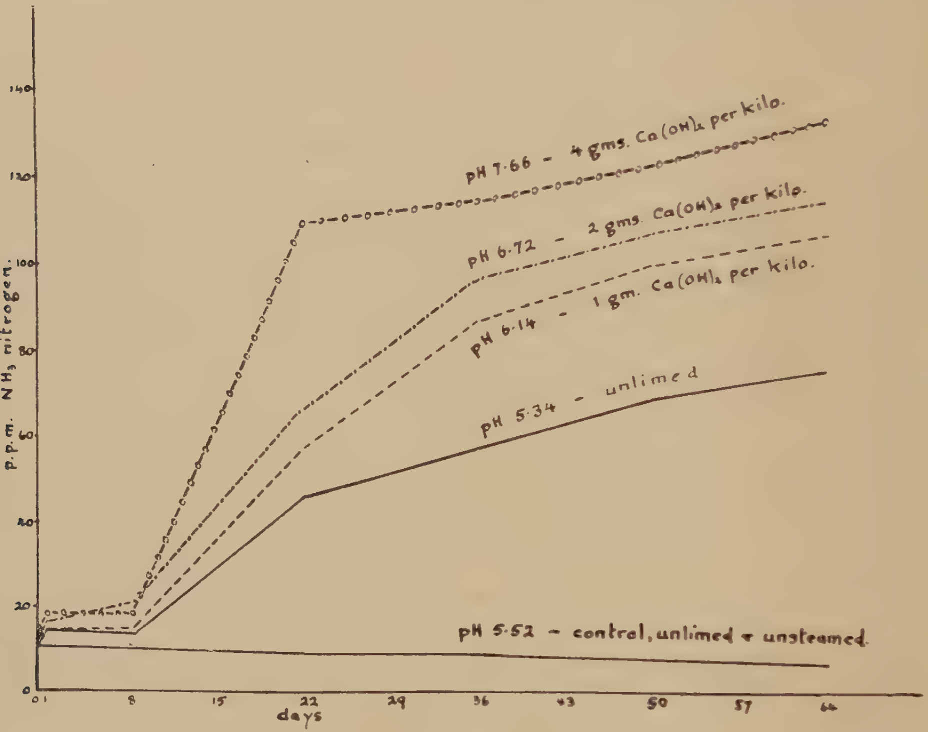

FIG. 4.

The effect of lime on ammonia production in steamed soils (Soil 4).

The experiment was repeated on another less acid soil (soil 4) with the following analysis:

pH	5·52
Loss on ignition	8·63%
Total nitrogen	0·26%

In view of the higher initial pH the quantities of hydrated lime were reduced as follows: 1 gram/kilo, 2 grams/kilo and 4 grams/kilo. The results are plotted in Fig. 4 and are similar to those obtained with the previous soil although in this case the amount of ammonia produced in the unlimed soil was the least and higher quantities were produced for each addition of lime. Actually greater quantities of ammonia were produced in this soil but the initial retardation in ammonification was similar in both cases. There seems to be a slight initial rise after one day (probably due to chemical breakdown) and then at least a week passes during which ammonification is at a standstill. The most likely explanation of this phenomenon is that in these rather acid soils there are few if any ammonifying bacteria and it is possible that they do not survive the steaming. Ammonification on these acid soils will normally be carried on by fungi but steaming will kill them and only when the soil becomes reinfected with fungi or ammonifying bacteria or both, will ammonification proceed. If only a small number of ammonifying bacteria survive steaming (assuming that a few were originally present), it may take a little time for them to multiply and begin active ammonification. When the ammonification does start, it is clear that it proceeds much more rapidly on the soils which have been limed and, indeed, the higher the pH within the limits of these experiments the greater is the quantity of ammonia produced.

The effect of steaming on the water-soluble organic matter and water-soluble and exchangeable manganese.

Steaming has a marked effect on the coloration of water extracts of soils, the solution becoming yellow or even light brown in colour. Steaming for different lengths of time and at different pH levels gives solutions which are progressively darker with longer steaming and increasing pH. The colour is completely removed on oxidation by $KMnO_4$ and is obviously of organic origin. So far, it has not been possible to examine the nature of this water-soluble organic matter and it is important to do so in view of its possible toxic nature but some estimations have been made of the water-soluble organic matter oxidized by N/20 $KMnO_4$ (Table VIII).

TABLE VIII.

Water-soluble organic matter in soil, before and after steaming.

Soil.	ccs. N/20 $KMnO_4$/100 grams Soil.	
	Before steaming.	After steaming.
Centre 1	55	210
,, 2	113	143
,, 3	32	159
Peat Soil	301	2252

It is clear that steaming has a big effect on the amount of water-soluble oxidizable organic matter and, on keeping a steamed soil, the concentration slowly decreases. Some idea of the differences due to steaming at different pH values and the effect of storing is given in Table IX.

In view of the increase in water-soluble oxidizable organic matter on steaming, one might naturally expect to find some changes in the water-soluble and exchangeable manganese and these actually occur; the pH of the soil also has a great effect. In general there is a definite but not spectacular increase in water-soluble manganese on steaming, and the concentration slowly decreases on storing (Table I). This applies particularly to soils with a pH higher than 5·5. On more acid soils there are

TABLE IX.

Water-soluble organic matter in steamed soils of different pH values.

pH	ccs. N/20 $KMnO_4$/100 grams Soil	
	Immediately after Steaming.	5 weeks later.
5·4	99	50
6·2	109	81
7·0	117	84
8·1	144	116

generally large increases in the water-soluble and exchangeable manganese. If these acid soils are limed before steaming, however, there is a big reduction in manganese as shown in Table X.

TABLE X.

Reduction in the manganese content of an acid soil brought about by liming before steaming.

Treatment.	pH.	p.p.m. Water-Soluble manganese in soil.		p.p.m. Exchangeable manganese in Soil.	
		13.10.44.	23.11.44.	13.10.44.	23.11.44.
Unsteamed	4·5	39	41	63	65
Steamed	4·6	99	53	179	139
Steamed, after addition of $Ca(OH)_2$	7·1	6·8	0·5	66	3·7

Clearly, if the soil is very acid, the water-soluble and exchangeable manganese increase markedly on steaming, but decrease when the soil is limed before steaming. It must be emphasized, however, that for less acid soils the effect is not so marked but nevertheless quite distinct and possibly of some practical importance.

DISCUSSION.

Whilst the primary reason for the steam-sterilization of soils is the destruction of pathogenic organisms, it is clear that many other changes are brought about. Some of these secondary effects are initially harmful in many soils but in general the process as a whole is eventually beneficial to plant growth. The temporary retardation of germination and early plant growth is dependent on many factors such as the plant species, soil type and environmental conditions. It is generally accepted (1, 5) that ammonia, the production of which is usually greatly increased by partial sterilization, is the most likely toxic agent but the break-down products of the organic matter may also be partly responsible (6) as may the increase in water-soluble or exchangeable manganese.

So far as we know, the chemical changes effected by steam sterilization in actual practice have not been studied and neither have various other matters of practical importance such as the length of time of steaming, the soil moisture content and the soil pH, which may have considerable effect on the subsequent changes. It is probably best to examine first the results of the laboratory experiments. It is clear from Figs. 1 and 2 that the length of time for which steam is passed has little effect on the total production of ammonia. Steaming at 212° F. for half an hour or more, as compared with steaming for shorter periods, causes an initial increase in the quantity of ammonia produced and, for one week thereafter, a retardation. Five minute steaming is just as efficient as longer steaming in its effect on ammonification and, if this were a desirable feature, as it is in some instances in practice, there appears to be a case for passing steam for as short a time as possible consistent with the destruction of harmful organisms. Retardation in plant growth is sometimes attributed to over-heating or passing steam for a longer time than is usual. It would certainly seem that some chemical breakdown is brought about by long steaming and the possible toxic nature of some of these break-down products must be borne in mind. On the question as to whether ammonia is responsible for retardation, it is interesting to note that, if anything, slightly less ammonia is eventually produced by longer steaming.

Figs. 1, 2, 3 and 4 indicate that, after steaming, ammonification proceeds rapidly for a period of about five weeks, the process gradually slowing down and reaching a maximum after about two months. It is not possible to say at present whether ammonification would proceed further if ammonia were being removed by nitrification or by growing plants, but there is evidence (Table IV) that ammonification comes to a standstill in bottled soils even on the resumption of nitrification.

In our experience the ammonia concentration in most glasshouse soils before steaming is in the neighbourhood of 10 to 15 p.p.m. ammonia nitrogen on the oven-dry soil basis and the concentration, one to two months after steaming, reaches approximately 50 to 150 p.p.m., depending on various factors. At the same time the nitrate concentration may be anything from 50 to 250 p.p.m. nitrate nitrogen. The total ammonia and nitrate nitrogen in steamed soils might therefore vary between approximately 100 to 400 p.p.m. which is equivalent to about 1,000 to 4,000 lb. sulphate of ammonia per acre. It is not difficult to attribute retardation in germination and in early plant growth to this high available nitrogen concentration, especially at the upper level.

The practice of flooding after steaming is often recommended and the reason for this is not fully understood. It is clear from these results that little ammonia will be leached from the soil if it is flooded soon after steaming because ammonification proceeds for a few weeks thereafter. There will probably be a considerable loss of nitrate and much of the water-soluble organic matter and manganese will also be leached out. One might infer from this that if the toxic agent is removed by flooding, it can scarcely be the ammonia which is responsible. One of the undoubted benefits of flooding is the thorough wetting of the sub-soil.

Another important practice, especially in the use of sterilized soil for seed and potting composts, is to leave the soil for three to six weeks to " recover ", the general belief being that some of the nitrogen is lost. The results of all these experiments show, however, that far from nitrogen being lost, the available nitrogen content continues to increase rapidly for about five weeks. In the laboratory experiments it was the ammonia content only which increased but it can be expected in practice that nitrification will set in during this period and, at the end of three to six weeks, the ammonia content will probably be normal. At the same time, the water-soluble organic matter and manganese which may be toxic, are also decreasing in concentration. It is difficult, therefore, to decide from these experiments whether ammonia, toxic organic matter, or manganese is responsible for retardation in plant growth. One instance of retardation in potted tomato plants came to our notice. Some of these plants had been liberally watered and were growing well; the others were very much retarded. The soil from the good pots contained 32 p.p.m. ammonia nitrogen and 13 p.p.m. nitrate nitrogen and, from the affected pots, 350 p.p.m. ammonia nitrogen and 215 p.p.m. nitrate nitrogen. Unfortunately the water-soluble organic matter and manganese were not estimated. With available nitrogen at the level found in these latter pots, retardation might well be expected.

While discussing the question of retardation, it is instructive to examine the effect of steaming limed soil. Lawrence and Newell (2) have shown that the addition of lime to a soil before steaming may have a serious retarding effect. Our experiments show that, after a week's delay, ammonification proceeds rapidly on limed soils and the amount of ammonia finally produced increases with each addition of lime. It is not possible to say definitely that the retardation is due to the ammonia, however, as the water-soluble organic matter also increases. The water-soluble and exchangeable manganese contents are smaller in soils limed before steaming and it would thus appear that manganese cannot be responsible for all cases of retardation. It is highly probable that retardation may be due to one or all of the three factors, ammonia, toxic organic compounds or manganese, depending on the soil conditions. The first two factors generally go hand-in-hand and will be greatest in rich soils of high pH, whereas toxic manganese will be greatest in steamed soils of low pH.

In two cases of retardation recently investigated, tomato seedlings were showing the symptoms of induced iron deficiency and had manganese contents of 1,850 p.p.m.

If it is desired to keep the production of available nitrogen as low as possible and reduce the risk of retardation to a minimum, it would appear desirable not to manure for some time before steaming. The soil, too, should not be limed before steaming, neither should it be very acid; it should be as dry as is consistent with the efficiency of destruction of pathogenic organisms.

Under these conditions the minimum amount of available nitrogen, water-soluble organic matter and manganese will be produced and, by the judicious use of manures afterwards, it should be possible to exercise a considerable measure of control over the subsequent production of available nitrogen and plant-growth.

SUMMARY.

1. Some of the chemical changes following steam sterilization have been examined both in commercial glasshouses and in laboratory experiments.

2. Changes in the ammonia concentration, water-soluble organic matter, water-soluble manganese, and pH have been followed.

3. The production of ammonia in steamed soils of varying moisture contents and of varying pH as well as in soils steamed for various times, has been estimated over a period of some weeks.

4. Steaming soils of high moisture content and high pH leads to the production of larger amounts of ammonia than does steaming under drier and more acid conditions. The length of time of steaming has little effect on the production of ammonia.

5. Steaming leads to an increase in water-soluble and exchangeable manganese, especially in acid soils.

6. Liming a soil before steaming has a very marked effect on the production of ammonia and water-soluble organic matter.

7. The possible toxic effects of ammonia, water-soluble organic matter and manganese on plants are discussed. The conditions of steaming, likely to reduce these possible effects to a minimum, are defined.

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THE INFLUENCE OF SOME GENETIC AND ENVIRONMENTAL FACTORS ON THE CONCENTRATION OF *L*-ASCORBIC ACID IN THE TOMATO FRUIT

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SYNOPSIS

Rainfall and fluctuations in shade temperature do not influence the concentration of vitamin C in the tomato fruit whereas illumination does exert an influence, probably due to the nature of the actinic composition of the light reaching the plant. Tomatoes growing in the sun contain more vitamin C than do those growing in the shade. This applies to fully grown green fruits as well as to fully grown red fruits, but under the same conditions of illumination the vitamin C content is higher in the latter. Fully grown green tomatoes raised in the sun may even contain more vitamin C than do fully mature red tomatoes grown in the shade. Tomatoes ripened off the plant late in the season apparently retain their vitamin C content unchanged during storage. Varietal differences in the vitamin C content cannot be demonstrated in tomatoes grown under conditions employed in practice. Tetraploid tomatoes grown in the shade contain more vitamin C than do diploid tomatoes similarly grown. When both are grown in the sun, the differentiation is not so marked.

The investigation of the influence of environment and genetic constitution on the formation of *L*-ascorbic acid in the plant is associated with many difficulties because of the number of unknown factors which cannot be controlled experimentally and which are involved in the process. It is therefore not surprising that much disagreement on the subject exists in the literature. Although these difficulties cannot be fully overcome with our present knowledge of the subject, a number of crucial facts bearing on the problem can nevertheless be established by controlling as much as possible the experimental conditions and by repeating the experiments until the reproducibility of the observations is established. Such an investigation must necessarily be of long duration since the experimental material becomes available only once a year.

The present contribution correlates certain genetic and environmental factors with the concentration of *L*-ascorbic acid in the tomato fruit. These experiments were carried out in 1940, 1941, 1943, 1945 and 1947. Reliable experimental material was not available in 1942 and 1944 owing to enemy action and other wartime difficulties.

METHODS.

The seeds to produce the plants grown under glass were sown in John Innes Seed Compost on 9th February each year. The seedlings were transferred to boxes on 18th February and to 3 in. pots on 11th March in John Innes Potting Compost;

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they were planted out in the glasshouse on 7th April. Subsequently the plants were top dressed with fertilizers as required. The seeds to produce the plants grown out of doors were sown in John Innes Seed Compost, during the first half of April, in 1940 and 1941 in the first week and, in the other years, in the second week. The treatment of the seedlings was the same as of those grown under glass. In all years the plants were planted in the open ground the last week in May. In the plots in the open, a mixture of equal parts of composted straw and farmyard manure was applied at the rate of 30 tons per acre and dug in during the winter months. In May, two weeks before planting, hoof and horn meal was applied at the rate of 8 oz. per square yard. No potash or phosphate was applied because analysis of the plots showed that supplies of both were adequate. About the middle of the growing season, mid-July, a top dressing of dried blood, 4 oz. to the square yard, was applied to the plots and hoed in. The plants exposed to the sun were grown on a border facing south. Those grown in the shade were situated on a border facing north, close to a wall 7 ft. 6 in. high, and the plants were screened from west and east to keep the sun from them.

The samples of tomatoes were collected from no more than two trusses at any one time, and as the season progressed they were accordingly taken from higher parts of the plant. In 1940 and 1941 the fruits were sent by post for chemical examination at East Malling, where they arrived the following day. During the other seasons they were sent to the Lister Institute and arrived there on the day of picking. On its arrival, the material was placed in a refrigerator at −20° C. and the juice was prepared from the frozen fruit the following day, as described by Sansome and Zilva.* In the case of trichloroacetic acid extractions, an equal volume of a 10 per cent. solution of the reagent was used with the entire frozen fruit which was ground in the same way as in the extraction of the juice.

The only really specific method for the determination of *L*-ascorbic acid is the biological assessment. This procedure is, however, very laborious, time-consuming and costly, and the use of it in the present investigation, which entailed a great number of analyses, would have been associated with almost insuperable difficulties. Furthermore, the accuracy of the biological method is not very great and consequently only very marked differences between the various samples could be detected in this way. Fortunately it was found (Sansome and Zilva, 1933) that the capacity for reducing indophenol and the biological response of tomato juice corresponded with those of genuine *L*-ascorbic acid. In consequence figures obtained by the indophenol method were considered dependable, at least within the limits of accuracy of the biological method.

In recent years two procedures were developed which made chemical tests for *L*-ascorbic acid more specific. One is based on the principle of the differential rates of combination of *L*-ascorbic acid and some other inactive indophenol-reducing substances which simulate *L*-ascorbic acid, with formalin at pH 1·5 and at pH 3·5 (Lugg, 1942); the other is based on the red coloration given by the 2 : 4-dinitrophenylhydrazine derivative of dehydroascorbic acid with 85 per cent. sulphuric acid (Roe and Kuether, 1943). Even these tests cannot be considered as absolutely specific for *L*-ascorbic acid (*vide* Snow and Zilva, 1944; Penney and Zilva, 1945). Nevertheless they were utilized in an attempt to throw further light on the specificity of the various chemical tests for *L*-ascorbic acid in tomato juice. A great number of determinations was accordingly carried out simultaneously on the same material: (1) by titration with indophenol of the juice directly and after treatment with H_2S—the reduction was necessary as the juices from immature fruits contained considerable quantities of dehydroascorbic acid; (2) by titration with indophenol of trichloroacetic extracts treated with H_2S; (3) by a modified Roe and Kuether method (Penney and Zilva, 1945); (4) by a modified Lugg method (Snow and Zilva, 1944). A statistical analysis of the figures obtained by the various procedures showed an agreement of about 10 per cent. This suggested that figures obtained by any one of these methods are in all probability those of genuine *L*-ascorbic acid especially because, as already mentioned, the biological method showed that the reduction of indophenol in tomato juice was in the main due to vitamin C. Consequently all our results are accurate at least within the above limit.

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* To each 50 gm. of the frozen tomato, 20 gm. of sand were added and the mixture finely ground. After squeezing through muslin, the residue was well ground again. The squeezed-out juice was then added to the ground residue, ground once more and again pressed out through muslin. This was repeated a third time. In calculating the results in these estimations and in those of the trichloroacetic acid extracts, it was assumed that 10 per cent. of the original fruit consisted of inactive solids and allowances were made accordingly.

RESULTS.

Vitamin C content of different varieties of tomatoes.

The object of these experiments was to ascertain whether the antiscorbutic activity of tomatoes grown out of doors and under glass varied with the variety. The work was carried out in 1940 and 1941. In the former year the tomatoes grown in the open were picked on the 20th August and again on 20th September, and the fruit grown under glass on 9th July. In 1941, six samples of most of the varieties were collected between 11th September and 9th October in the open and five samples between 6th August and 24th September under glass. The vitamin C determinations in these experiments were carried out by indophenol titration of the juice. The results are summarized in Table I.

The figures obtained with the fruits grown out of doors and under glass in both years were analysed statistically by calculating the " t " values (Snedecor, 1937). The general conclusion drawn was that although some varieties appeared definitely more active than others when grown one year, this did not hold true when the same varieties were grown another year. The activity of the same variety sometimes even varied with the time of the season when it was picked. Thus Potentate, Sunrise, Best of All and Kondine Red grown under glass in 1941 were found to be significantly higher in their vitamin C content than the other varieties grown that year under the same conditions. Yet in 1940 Potentate and Sunrise also grown under glass were found to be two of the least active varieties. Furthermore, in the same year the varieties mentioned above when grown out of doors were again found to be the highest when collected on 20th August. This superiority was not, however, maintained by all of these varieties later in the season (20th September). Our results, therefore, taken in conjunction with those obtained by Baker and Parkinson (1947), show that it would not be justified to differentiate in a general way between the activities of the different varieties. It is, of course, not ruled out that if it were possible to carry out experiments under absolutely identical conditions such a differentiation might be found to exist. With our present knowledge of the subject, however, such an investigation could not be easily controlled and any conclusions formed would be open to criticism.

The results also show beyond doubt that tomatoes grown out of doors possess a higher vitamin C content than those grown under glass and Currence's (1939) observation is thus once more fully confirmed.

TABLE I.

L-ascorbic acid content (mg./100 g.) of different varieties of tomatoes.

Variety.	1940.			1941.	
	Grown under glass 9th July.	Grown out of doors.		Grown under glass 11th September-9th October.	Grown out of doors 6th August-24th September.
		20th August.	20th September.		
Potentate	10·8 (4)	25·5 (5)	31·0 (4)	15·6(15)	27·1 (18)
S.D.	1·04	2·7	4·28	3·23	5·53
Sunrise	10·3 (5)	22·2 (5)	33·8 (5)	14·3 (20)	24·1 (23)
S.D.	1·68	3·02	3·16	3·05	5·19
Best of All	11·4 (4)	24·3 (4)	31·6 (5)	14·7 (17)	22·5 (21)
S.D.	0·93	2·94	3·13	2·35	4·21
Kondine Red	11·2 (5)	22·2 (5)	32·0 (5)	14·3 (19)	22·4 (14)
S.D.	1·53	5·03	3·38	3·76	5·69
Buckley	11·2 (5)	21·0 (4)	28·8 (5)	—	—
S.D.	2·03	3·13	4·27	—	—
Cracker Jack	14·0 (4)	21·8 (5)	26·7 (4)	11·4 (16)	21·9 (6)
S.D.	2·52	1·16	6·58	2·03	5·78
Plumpton King	12·5 (5)	19·9 (5)	35·7 (5)	11·8 (18)	22·0 (20)
S.D.	1·20	1·51	8·23	2·33	5·61
Radio	11·3 (5)	22·0 (5)	31·2 (5)	13·0 (19)	21·5 (8)
S.D.	2·99	3·94	2·84	3·51	5·0
Kondine Red ×Dpsory	17·1 (5)	17·7 (5)	36·0 (5)	—	—
S.D.	3·1	2·21	2·85	—	—
Ailsa Craig	16·8 (5)	24·1 (5)	32·3 (5)	—	—
S.D.	2·35	1·79	3·36	—	—
Moneymaker	—	—	—	13·4 (16)	20·3 (5)
S.D.	—	—	—	4·20	2·43
Mean of all varieties	12·6 (47)	22·0 (48)	32·5 (48)	13·5 (140)	23·1(116)
S.D.	2·76	3·4	4·93	3·26	5·4

Glass house temperature: 60-70° F.
Figures in brackets indicate the number of fruits from which the average was obtained.
Dpsory is an unnamed multiple recessive form with yellow fruits. All the others have red fruits, but they differ from one another in other genetic characters.
S.D. = Significant difference.

The influence of light during growth on the concentration of L-ascorbic acid in the tomato fruit.

These experiments were carried out in 1943, 1945 and 1947. Fully grown tomato fruits, both ripe and green (except 1945), were collected from plants grown in the sun and shade simultaneously at various periods of these seasons and the analytical results were co-ordinated not only with the sunshine records for these years, obtained from the Royal Observatory at Kew, but also with the temperature and rainfall recorded at the John Innes Institution. The results are represented graphically in Figs. 1, 2 and 3.

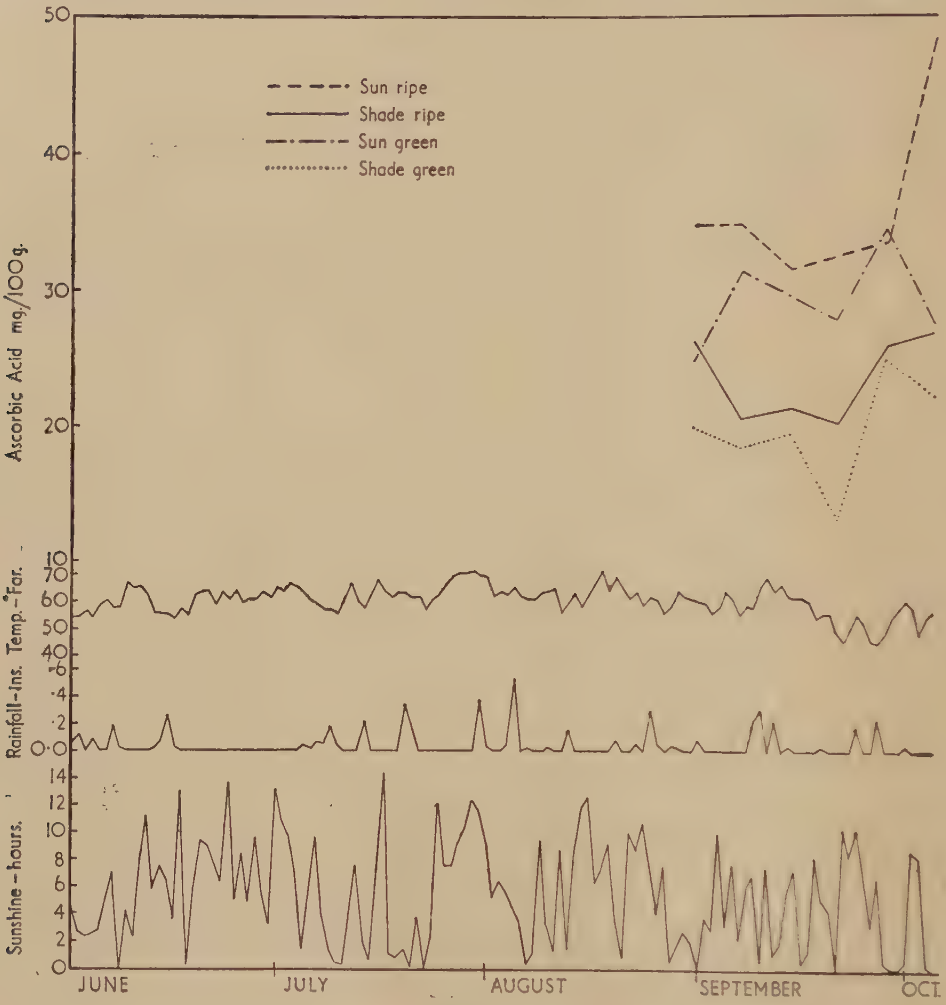

Fig. 1.

1943.

Season 1943. The plants were transplanted in the ground 29th May and 1st

June and the period during which the various collections were made was between 31st August and 5th October. During the pre-harvest period the total rainfall was 3·56 in., the mean temperature 62° F. and the total sunshine 528 hours. During the harvest period these figures were 1·16 in., 56° F. and 177 hours respectively. One determination on two or three pooled fruits from each of the following varieties: Sunrise, Kondine Red and Cracker Jack, was carried out in each picking and the average figures of these results are plotted on the graph. The analyses were carried out by titrating the trichloroacetic acid extracts after reduction with H_2S.

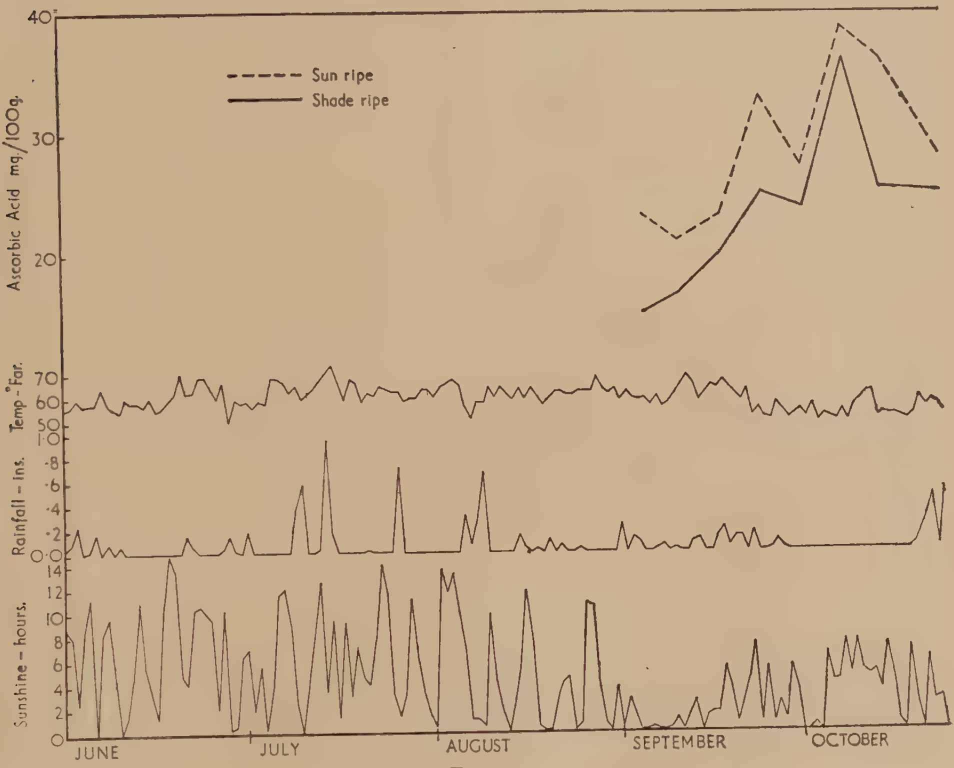

FIG. 2.

1945.

Season 1945. These experiments were carried out only on red ripe tomatoes of the variety "Sunrise" grown in the sun and in the shade. The plants were transferred to the ground on 28th May and the picking period began on 5th September. Rainfall was 5·97 in., mean temperature 61° F. and the total sunshine 473 hours for the pre-harvest period and 2·07 in., 56° F. and 138 hours respectively for the harvest period. The analytical technique was similar to that of 1943, five fruits of each picking were analysed and of these the average figure is plotted on the graph.

Season 1947. Variety "Sunrise" was again utilized in these experiments. The plants were transplanted in the ground on 1st June and the fruits were collected between 20th August and 23rd September. Rainfall was 3·56 in., mean temperature 63° F. and the total sunshine 456 hours during pre-harvest and 1·36 in., 63° F. and 223 hours respectively for the harvest period. The analyses were carried out by the modified Roe and Kuether method. Six fruits of each sample were analysed and of these the average figure is plotted on the graph.

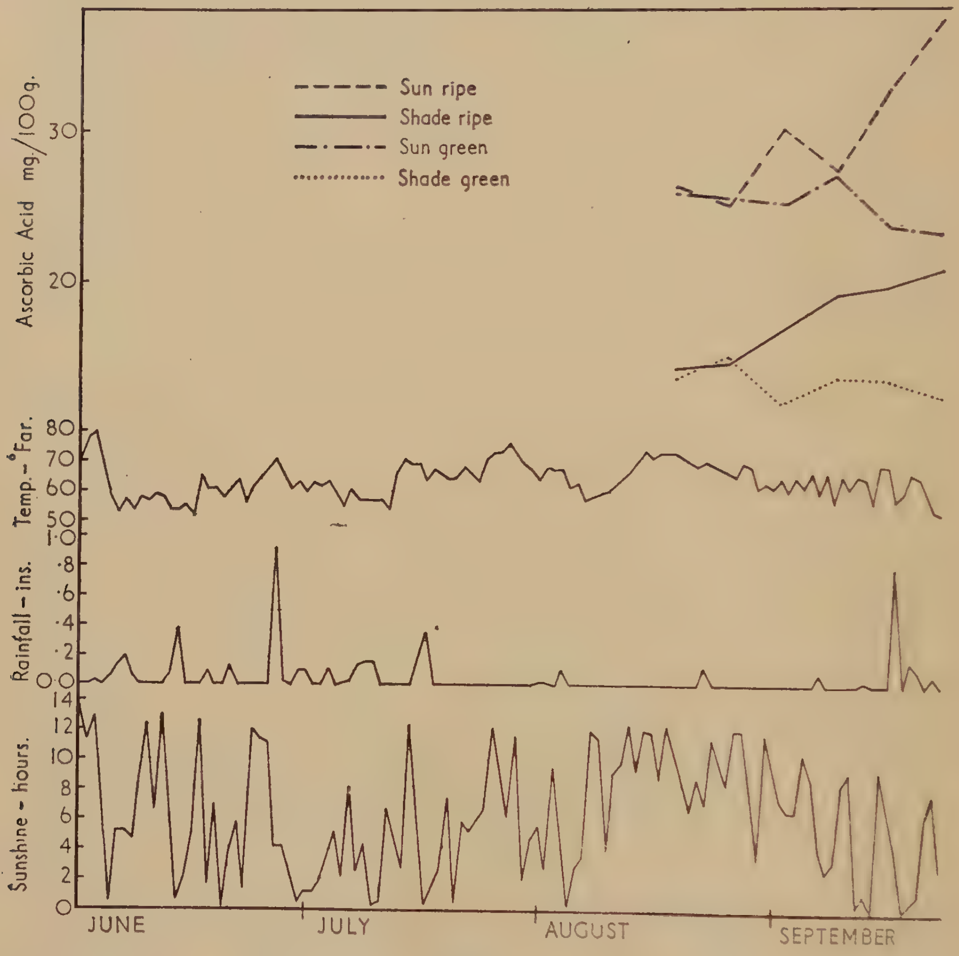

FIG. 3.

1947.

Little doubt can exist that the ripe tomatoes of 1943 and 1947 grown in the sun had a higher *L*-ascorbic acid concentration than those grown in the shade. The difference is not so high in 1945. A statistical examination of the figures, however, showed that this difference was significant. Thus the means of the different pickings of the ripe fruits grown in the sun ranged from 20·8 mg./100 g. to 38·6 mg./100 g.

TABLE II.

Average L-ascorbic acid content of various pickings of tomatoes correlated with the hours of sunshine of the preceding week.

1943.				1945.			1947.			
Date Picked.	Total Sunshine in preceding week. Hours.	Ascorbic Acid Content mg./100g.		Date Picked.	Total Sunshine in preceding week. Hours.	Ascorbic Acid content. mg./100g.	Date Picked.	Total Sunshine in preceding week. Hours.	Ascorbic Acid Content mg./100g.	
		Ripe.	Green.			Ripe.			Ripe.	Green.
31st Aug. ..	40·6	34·7	24·4	5th Sept. ..	22·0	26·4	19th Aug. ..	76·4	26·1	23·6
7th Sept. ..	24·3	34·2	29·0	11th ,, ..	1·9	20·8	26th ,, ..	63·0	25·2	25·6
14th ,, ..	31·4	31·5	24·7	18th ,, ..	7·4	22·9	2nd Sept. ..	65·9	30·1	25·2
21st ,, ..	29·3	34·0	29·3	25th ,, ..	25·6	32·8	9th ,, ..	46·5	27·3	27·1
28th ,, ..	42·4	38·5	35·5	2nd Oct. ..	18·7	29·4	16th ,, ..	31·6	32·7	23·7
5th Oct. ..	24·4	48·5	48·5	9th ,, ..	15·1	38·6	23rd ,, ..	27·2	37·4	23·2
				16th ,, ..	37·1	35·8				
				23rd ,, ..	21·1	29·5				

with a mean for the season of 28·3 mg./100 g. juice, S.D.,* 7·07; those of the ripe tomatoes grown in the shade ranged from 15·1 mg./100 g. to 36·1 mg./100 g. with a total mean of 22·8 mg./100 g., S.D., 6·44.

The point to be particularly noted is that the beneficial influence of the sun on the *L*-ascorbic acid concentration of the tomatoes extends also to the green fruit to such an extent that the content of the ripe tomatoes grown in the shade can be under certain circumstances lower than that of green fruit grown in the sun. It is therefore not surprising that disagreement exists as to whether ripe tomatoes contain more *L*-ascorbic acid than green tomatoes. [Cf. Maclinn, Fellers and Buck (1936); Murphy (1942); Baker and Parkinson (1947).] Our results show that under similar conditions of illumination this is definitely the case.

It appears that the hours of sunshine during the period preceding the harvesting of the tomatoes have no influence on their *L*-ascorbic acid content. This can be seen from the following figures. In 1943, the average of hours of sunshine per day was 5·7, the ascorbic acid concentration of the first picking was—(ripe) 34·7 mg./100 g., (green) 24·7 mg./100 g. In 1945, the average of hours of sunshine per day was 4·9, the *L*-ascorbic acid concentration of first picking was—(ripe), 23·0 mg./100 g. In 1947, the average of hours of sunshine per day was 5·6, the *L*-ascorbic acid concentration of the first picking was—(ripe), 26·3 mg./100 g. (green) 26·0 mg./100 g. The graphs show further that there is no relationship between the hours of sunshine immediately preceding the harvesting period and the concentration of *L*-ascorbic acid in the fruit. Above all, as will be seen from Table II, this is also true for the period during which the tomatoes have been picked. Hamner, Bernstein and Maynard (1945), and Somers, Hamner and Nelson (1945), concluded from their investigations that the amount of sunshine for 18 days prior to harvest is closely correlated with the *L*-ascorbic acid content under field conditions. That is not indicated by our results. It would appear that the hours of sunshine bear no quantitative relation to the *L*-ascorbic acid content in the fruit during the solar illumination. Another factor is evidently involved, probably the transparency of the atmosphere. Rainfall and shade temperature may be excluded as factors influencing the concentration of *L*-ascorbic acid in the tomato fruit whether they are grown in the sun or in the shade.

The influence of ripening of green tomatoes off the plant on the L-ascorbic acid content of the fruit.

Fully grown green tomatoes were picked at the end of the season in 1943, 1945 and 1947 and ripened off the plant in the glasshouse (range of temperature 60 to 70° F.). The red tomatoes thus ripened were then analysed by the methods mentioned for the respective years in the preceding sections. The results are summarized in Table III.

These results indicate that no very significant change, if there were any change at all, took place in the ascorbic acid content when the green mature tomatoes collected at the end of the season were ripened off the plant and that this holds true whether the tomatoes were grown in the shade or in the sun. Although there was a rise in the case of the green fruit, grown in the sun, picked in 1945, this was not so in 1947. In fact the tomatoes picked on 1st October of that year showed

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* S.D. = Standard deviation.

Table III.

L-ascorbic acid content of tomatoes ripened off the plant.

Season.	Date when picked.	Date when ripened.	Concentration of Ascorbic Acid mg./100g. (averages).			
			Grown in Sun.		Grown in shade.	
			Green fruit on picking.	Ripened red fruit.	Green fruit on picking.	Ripened red fruit.
1943 ..	26th Oct.	16th Nov.	—	—	18·7(6)	21·2(6)
1945 ..	23rd. Oct.	30th Oct.	25·5(5)	32·2(5)	23·9(5)	27·8(5)
		6th Nov.	25·5(5)	29·9(5)	23·9(5)	24·1(5)
		13th Nov.	25·5(5)	30·0(5)	23·9(5)	25·5(5)
		20th Nov.	25·5(5)	33·6(5)	23·9(5)	22·1(5)
		27th Nov.	25·5(5)	29·4(5)	23·9(5)	17·3(5)
				Mean 31·0		Mean 23·4
1947 ..	23rd Sept.	8th Oct.	23·6(6)	25·1(8)	12·1(6)	15·5(8)
		16th Oct.	23·6(6)	22·1(8)	12·1(6)	13·7(8)
				Mean 23·5		Mean 14·6
	1st Oct.	16th Oct.	31·6(6)	22·6(8)	15·2(5)	15·8(7)

Figures in brackets indicate the number of fruits from which the average was obtained.

a fall in the *L*-ascorbic acid content on ripening. Such fluctuations, in all probability, were due to factors not easily controlled. A marked rise or fall in the content would nevertheless have been reflected in spite of these variations.

The L-ascorbic acid content of diploid and tetraploid tomatoes grown under comparable conditions.

Sansome and Zilva (1933) have shown that tomato fruits from tetraploid strains contained about twice as much *L*-ascorbic acid as the fruits of the diploid strain from which they are derived. In pursuing the investigation, it was found (Sansome and Zilva, 1936) that this disparity in the *L*-ascorbic acid content was not always as marked as that first observed. It became therefore apparent that other factors were exercising an influence in this connection, a possibility which was foreshadowed in the original communication. For a number of years we endeavoured to throw some further light on this problem and in 1947 we succeeded in obtaining some relevant information.

The diploid stock of the variety "Sunrise", used in the 1947 experiments already described, was induced to form tetraploid shoots and the fruits from these plants were grown under conditions identical to those under which the tomatoes from the original diploid stock grew. These fruits were also analysed by the modified Roe and Kuether method and the results are given in Table IV. While there was no significant difference in the *L*-ascorbic acid content between the tetraploid and diploid tomatoes grown in the sun, whether they were picked in a ripe or green condition, there was, however, in the tetraploid fruits (both ripe and green), a vitamin content markedly higher than in the diploid tomatoes when both were grown in

TABLE IV.

Average L-ascorbic acid content of diploid and tetraploid tomato fruits (*mg.*/100 *gm.*)

Date picked.	Grown in Sun.				Grown in Shade.			
	Ripe.		Green.		Ripe.		Green.	
	Diploid.	Tetraploid.	Diploid.	Tetraploid.	Diploid.	Tetraploid.	Diploid.	Tetraploid.
19th Aug.	26·3	28·1	25·9	26·8	14·0	19·4	13·5	18·9
26th Aug.	25·2	—	25·6	—	14·6	—	14·6	—
2nd Sept.	30·1	28·0(7)	25·2	26·3(4)	16·5	—	11·8	—
9th Sept.	27·3	26·3	27·1	26·6	19·0	—	13·3	24·3(3)
16th Sept.	32·7	29·1	23·7	31·0	19·4	22·6(4)	13·2	22·0(4)
23rd Sept.	37·4	32·6	23·2	23·2	20·5	17·6(1)	12·1	16·7(4)
Mean ..	29·8	28·7	25·1	26·8	17·3	20·4	13·1	20·1
S.D. ..	5·93	5·03	3·23	5·42	3·56	3·12	1·84	3·69

The figures represent the average of six fruits.
When this is not the case the number of fruits are given in brackets.

the shade. It may be mentioned that when a comparison is made of the mean values of the respective ripe fruits picked on 19th August and 16th September, eliminating that of 23rd September because only one fruit was available, the difference between the mean values is even greater, i.e. mean for diploids 16·6 mg./100 g. against 20·6 mg./100 g. for tetraploids. Unfortunately, the yields of the tetraploid tomatoes grown in the shade were low, particularly for the ripe fruits, a fact which prevented the use of a larger number of fruits. The results of this single experiment, however, are significant and show that when the plants are deprived of direct sunshine during growth, the *L*-ascorbic acid content of tetraploid tomatoes is not so much influenced as is that of diploid plants.

It would be appropriate to consider here the size of the fruit in relation to the higher antiscorbutic activity of the juice of tetraploid tomatoes. McHenry and Graham (1935) suggested that the difference in the *L*-ascorbic acid content between tetraploid and diploid tomatoes observed by Sansome and Zilva (1933) was, at least in part, due to the smaller size of the tetraploid tomatoes, because according to them the vitamin was more concentrated in smaller than in larger fruits. Sansome and Zilva (1936) in analysing their original results showed that this could not have been the case in their experiments. Furthermore, Maclinn, Fellers and Buck (1936) working with diploids were unable to confirm that in smaller tomatoes the *L*-ascorbic acid content was higher than in larger fruits. This is also our experience from results already obtained but not published. In this investigation we found that whilst in the case of the ripe tomatoes there was no significant difference between the weight of the tetraploid fruit grown in the sun and in the shade (means 28·8 and 27·3 g. respectively) the *L*-ascorbic acid content differed significantly (means 28·7 mg./100 g. and 20·4 mg./100 g. respectively) which suggests that in tetraploid as well as in diploid tomatoes there is no constant relationship between size of fruit and *L*-ascorbic acid content. Size grading could not unfortunately be carried out owing to the great difficulty in obtaining a sufficient number of tetraploid fruits grown in the shade.

DISCUSSION.

If the effect of the difference in the temperatures to which the fruits are exposed in the sun and in the shade is excluded, the general results obtained in these experiments suggest that light composed of certain wave lengths is particularly associated, whether it be directly or indirectly, with the formation of *L*-ascorbic acid in the tomato plant. The appearance of lycopin on ripening does not seem to play any part in this formation. The fact that the tomatoes grown under glass contain less *L*-ascorbic acid than those grown in the open suggests further that light of the low wave length region of the spectrum, which would to a large extent be held back by the glass, is probably involved in the process. This would also explain to some extent our observation of the lack of quantitative relationship between the hours of sunshine and the *L*-ascorbic acid content of the tomato fruit, since not only the time of exposure but also the quality of the sunlight actually reaching the plant would determine the formation of the vitamin and this would, of course, depend on the transparency of the atmosphere—a variable factor particularly in this country. From the above considerations it may nevertheless be expected that tomatoes, grown in places where the hours of sunshine are usually long and the transparency of the atmosphere is good, would be richer in *L*-ascorbic acid than the tomatoes grown in environments not enjoying these advantages. If the assumption that the composition of the light is connected with the formation of *L*-ascorbic acid is right it would in consequence appear that diploid tomatoes are more influenced in this respect than tetraploid tomatoes because, as was seen, they form less *L*-ascorbic acid in the shade.

Ripeness does not have as marked an influence as light on the *L*-ascorbic acid content of tomatoes since it was found that fully grown green tomatoes may contain considerably more *L*-ascorbic acid when grown in the sun than fully grown mature red tomatoes grown in the shade. As the green tomatoes, ripened off the plant, did not show any significant increase or reduction in the *L*-ascorbic acid content in the process of ripening, it follows that it is possible to obtain tomatoes ripened artificially which would contain large quantities of *L*-ascorbic acid providing suitably grown green fruit is chosen for storage. It may be mentioned that this applies to tomatoes picked late in the season. We have obtained indications that it may not hold true for green tomatoes picked in the early part of August, a point which, if fully established, is of theoretical rather than of practical interest.

SUMMARY.

1. It was not possible to demonstrate a varietal difference in the *L*-ascorbic acid content of tomatoes grown under glass or in the open under conditions employed in practice.

2. There is a higher concentration of *L*-ascorbic acid in tomatoes grown in the sun than in those grown in the shade not only in the red, ripe fruits but also in those which are green and unripened.

3. Fully grown green tomatoes raised in the sun may contain considerably more *L*-ascorbic acid than fully mature red tomatoes grown in the shade. When

grown under similar conditions of illumination, however, the concentration of *L*-ascorbic acid is higher in the red fruits than in the green.

4. Tetraploid tomatoes grown in shade contain more *L*-ascorbic acid than diploid tomatoes similarly grown. The difference is not so marked when they are grown in the sun.

5. Rainfall and fluctuations in shade temperature do not influence the concentration of *L*-ascorbic acid in the tomato.

6. The reduction of indophenol by tomato juice, or by the trichloroacetic acid extract, after reduction with H_2S, the Roe and Kuether and the Lugg methods are specific for *L*-ascorbic acid in tomato juice to the extent of at least 90 per cent.

7. It is suggested that light of the short wave length region of the spectrum is probably involved in the formation of *L*-ascorbic acid in tomatoes, although the temperature to which the fruits are exposed cannot be excluded as a possible factor.

We should like to express our gratitude to the many workers in our laboratories for the assistance they gave us at various times during this investigation.

Since the completion of this communication a paper by Pollard, Kieser and Bryan (1948) came to our notice in which they also conclude that there is no quantitative relationship between the hours of sunshine and the *L*-ascorbic acid content of the tomato fruit.

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CITRUS GROWTH STUDIES

I. PERIODICITY OF ROOT-GROWTH AND TOP-GROWTH IN NURSERY SEEDLINGS AND BUDLINGS*

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DURING the years 1937 to 1940 the author carried out an intensive investigation on the root-growth, top-growth and fruit-growth of citrus, the results of which he has been unable to publish until now on account of his proceeding on active service. The phase dealt with in this paper concerns observations made on the periodicity of top-growth and root-growth of seedlings of rough lemon (*Citrus limon* (Linn.) Burman) and sweet orange (*Citrus sinesis* (Linn.) Osbeck) and of Valencia orange budlings on these stocks in the nursery, and on the subsequent initial top-growth after planting out.

LITERATURE.

That the yearly top-growth of the evergreen citrus tree occurs in a series of flushes, usually two major and from one to three minor ones, depending on the climatic conditions under which the tree is growing, has been known to citrus growers for many years. Crider (1927) states that in Arizona there is generally a major flush in the early spring, a second medium one in mid-summer and a third medium one in early autumn. Reed and MacDougal (1937) record only three flushes on bearing Navel orange trees in California in 1935. In Palestine, Cossman (1939) and others report four flushes on bearing Jaffa oranges, the major one in late winter, a second in mid-summer, a third in early autumn and a fourth minor one, in warm years only, in early winter.

It was the original and comprehensive work of Crider (1927) which first proved that citrus roots also have distinct growth and rest periods of rather definite duration, these periods being shown in his work as alternating with the periods of top-growth. Of the three root-growth periods observed during the year, the first, in early summer, was greatest and most intense, the second, after mid-winter, was relatively weak, while the third, in early autumn, was the longest but was not so great or so intense as the first. Waynick and Walker (1930) found in California that different species of citrus all had three root-growth cycles during the year, the periods being similar to those found by Crider but less definite. Monselise (1944), working in different orchards with various stocks and with trees treated by different cultural methods, generalizes that in Palestine the Shamouti orange has two main root-growth periods during the year, the first from early winter through till late winter and the second from spring throughout the summer but that the latter period is spaced with brief interruptions.

EXPERIMENTAL PROCEDURE.

The material used for obtaining the data herein discussed was drawn from a citrus nursery established on light, sandy loam soil with a gravelly decomposed granite subsoil. The area is subtropical in climate, with a summer rainfall and mild winter.

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From seed beds planted with seeds from single parent trees there were set out in nursery rows 700 selected rough lemon and 700 sweet orange seedlings. A third of each of these were budded with Valencia orange buds cut from a single parent tree. Normal nursery cultural practices were followed, irrigation necessarily being at far more frequent intervals throughout the year than those occurring in an established, bearing citrus grove.

From October, 1938, to January, 1940, five rough lemon seedlings and five sweet orange seedlings were lifted each week, each five being consecutive individuals in the rows. Five budlings on rough lemon and five on sweet orange stock were lifted fortnightly during the same period.

The trees to be lifted were well irrigated immediately prior to lifting, so that a maximum amount of the root system was removed with the plants without damage. Adhering soil was washed off the roots. Observations described hereunder were then made. The roots and tops were now pruned back and the trees planted in the open ground, spaced three feet apart, in rows six feet apart, in a manner similar to the planting of trees when an orchard is established. All the replanted trees, including those planted in previous weeks, were irrigated the same day, so that weekly irrigations were given for the seventeen-month period of observation and the trees were thus always well supplied with soil moisture.

OBSERVATIONS.

The interpretations of the observations made are presented graphically; lists of dates and figures tend to blur the true trends of the actual growth periods. The nursery root-growth and top-growth for seedlings and budlings of rough lemon and sweet orange stocks are all plotted in Fig. 1. In addition, other graphs allowing direct comparison were utilized.

Nursery root-growth.

The root-growth activity of the newly-lifted trees was recorded by giving an arbitrary value to each individual plant, depending on the number and thickness of newly-formed white roots observed. Growth of both primary and fibrous roots in these particular observations was recorded as one and no differentiation was made between initiation of new roots and elongation of existing roots. It was quite common for only one main root or one side of the root system to show activity, the rest being dormant; hence only a general index of activity could be obtained. The arbitrary values assigned were:

No growth	0
Isolated growth	1
General growth	3
General active full growth ..	4
General growth hardening ..	2

The total values for each group of five plants were plotted on one graph, and the following conclusions drawn:

(*a*) Periods of root-growth activity, both in initiation and duration during the year, may vary by several weeks from season to season.

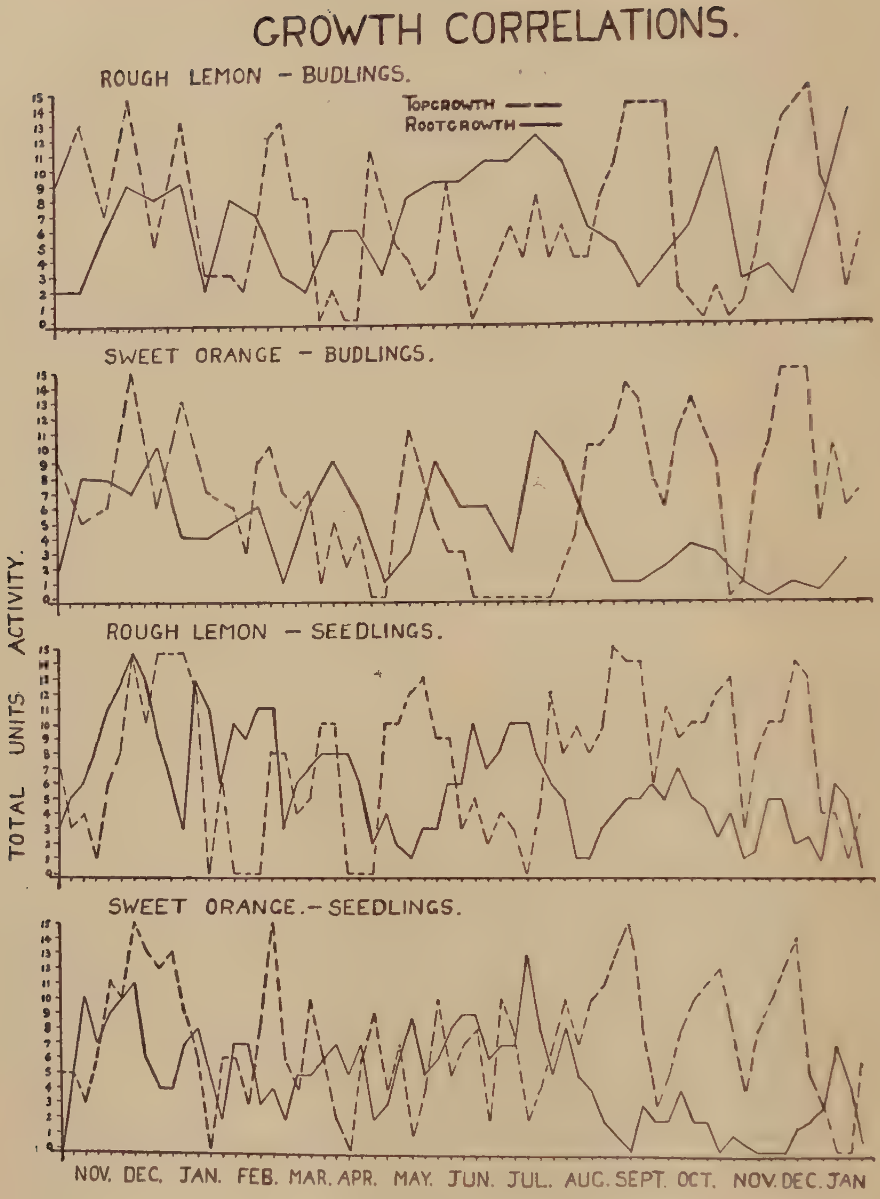

FIG. 1.

Root-growth and top-growth in nursery, expressed as units of activity during fifteen-month period.

(*b*) The general trend of activity was the same for *seedlings* and *budlings* with both rough lemon and sweet orange.

(*c*) Rough lemon showed a greater degree of activity than sweet orange.

(*d*) Some degree of root-growth activity was shown by rough lemon throughout the year, even during the rest periods, while sweet orange showed dormancy.
(*e*) Five periods of root-growth activity, with corresponding "check" periods between them, occurred during the year.

(i) The longest and most active period was, surprisingly enough, during the winter and it lasted from May to August with a sustained peak during June and July. This agrees with the findings of Monselise (1944) in Palestine on bearing trees, but differs from the findings of workers in Arizona and California where colder winters are experienced.

(ii) After a definite check period in September, there was a short period of medium activity during spring to early summer, especially with rough lemon but it was not so marked with sweet orange.

(iii) During the check period preceding the following major flush, the sweet orange was dormant. The time of initiation of this flush varied but was at its height in mid-summer, lasting about two months.

(iv) Preceded by an indefinite short check period, a minor flush occurred at the end of summer, lasting about a month.

(v) A more definite short check period preceded the mid-autumn minor flush, which also lasted about a month. This was followed by a definite check or rest period at the beginning of May and preceded the major winter flush.

Nursery top-growth.

At the time of lifting from the nursery, observations were made on the activity of top-growth of each individual and values assigned according to the stage of such growth. The relative amount of growth or degree of activity was not recorded.

Dormant	0
Flush starting	2
Full flush	3
Flush hardening	1

The total values for each group of five plants were plotted on one graph and the following conclusions drawn:

(*a*) Top-growth was an extremely variable factor to determine for an evergreen tree such as citrus when young and growing in a subtropical summer rainfall area, especially when receiving good cultural treatment for fertilization and irrigation.
(*b*) The general trend of activity was the same for *budlings* and *seedlings* and for rough lemon and sweet orange throughout the year but was not so marked as with root-growth.
(*c*) Rough lemon tended to be more definite than sweet orange in the growth periods which occurred and also reflected more dormancy during the check or rest periods. This is the opposite to what was found with root-growth.
(*d*) Four periods of top-flush and activity, two definite and two indefinite, with corresponding check periods between them, were observed ((i)-(iv) below). It is noted that five root-growth periods for the same plants were observed.

(i) The first major flush, which corresponds to the blossom flush of bearing trees, occurred in spring and lasted from August to October.

(ii) The following flush, also a major one, was at its height in mid-summer and lasted from mid-November to January.

(iii) The February-March flush was a minor one, indefinite in its intensity and duration.

(iv) The winter flush, which apparently does not occur on bearing trees in colder climates, was also a minor one and of great irregularity, but petered out towards July.

Correlation between root-growth and top-growth.

As a result of the findings of earlier workers it has been accepted as a basic physiological fact for citrus that, in general, during the time when the roots are actively growing, the shoots are quiescent and *vice versa*. In Fig. 1 are presented the curves for root-growth and top-growth activity during a continuous fifteen-month period for the same individuals of each group, allowing a direct comparison. From a study of these curves the following conclusions are drawn :

(*a*) With young nursery *seedlings* and *budlings*, adequately supplied with soil moisture, a trend in general towards alternation in periodicity of root-growth and top-growth existed, such trend becoming more definite as the plants grow older.

(*b*) The alternation in periodicity was more marked with budlings than with seedlings.

(*c*) Rough lemon and sweet orange did not show any basic difference in these correlations.

(*d*) Variations from the general trend were sufficiently frequent and large to cast doubt on this alternation as a basic physiological characteristic of citrus. Under stronger seasonal variation in climate it might be more pronounced but under mild, subtropical climates the principle is held to be not proven.

Top-growth of transplanted trees.

Since the main object of planting out trees throughout the year was to determine at which period the most favourable " take " resulted, observations on top-growth were confined to date of initiation of new growth, no record of degree of activity being kept. The average times, in weeks, between date of lifting and when four out of the five individuals in each group had shot, are presented as graphs in Fig. 2. From these graphs and from the data obtained the following conclusions are drawn :

(*a*) The general trends for rough lemon and sweet orange seedlings and their budlings were similar.

(*b*) Budlings on rough lemon stock made top-growth sooner, throughout the year after planting out, than did sweet orange budlings. No similar sustained difference was noted in the seedlings.

(*c*) From the end of July to the end of March *seedlings* took, on an average, from two to six weeks to shoot, with individual variations from one to seventeen weeks, the most favourable time being the beginning of November. From April to July the average period varied from six to nineteen weeks, with individuals taking up to twenty-nine weeks to shoot, the most unfavourable period being May and June.

(*d*) From the end of July to the end of March, *budlings* on rough lemon stock averaged from two to eight weeks after planting before they shot, while budlings on sweet orange stock over the same period averaged from four to thirteen weeks. The most favourable period was November-December for both stocks. Individual variations during this period were from two to ten weeks for rough lemon, and from two to forty-five and fifty-one weeks for sweet orange. From April to July the average period varied from nine to fourteen weeks for rough lemon, and from eight to nineteen weeks for sweet orange. The most unfavourable period was May-June for rough lemon, with individuals taking up to twenty-two weeks to shoot, and from March to June for sweet orange, many individuals taking from twenty-three to thirty weeks to shoot.

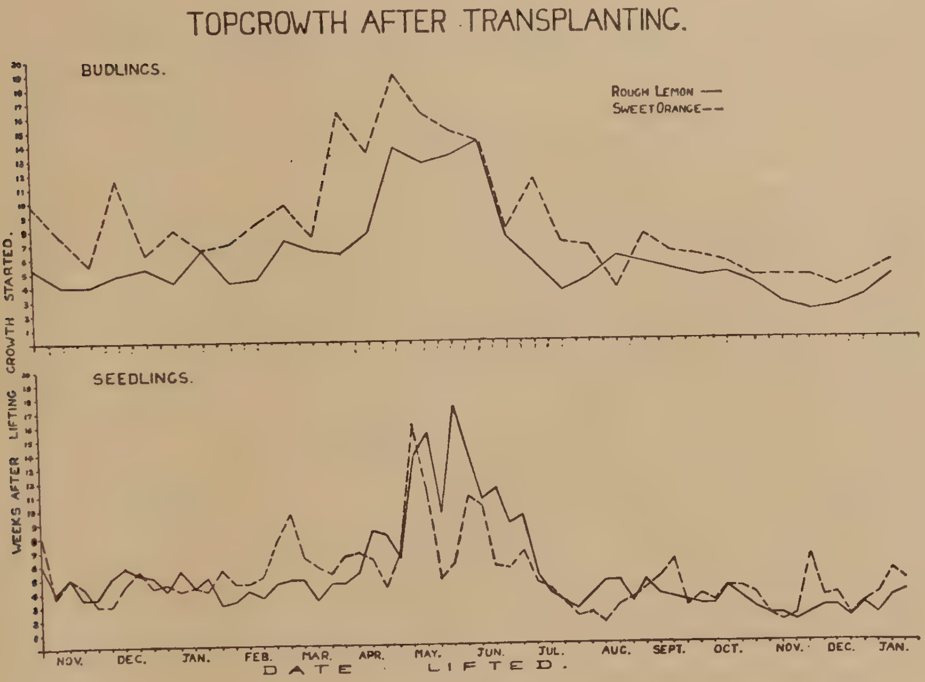

FIG. 2.

Top-growth in open ground after transplanting.

(*e*) When first top-growth has hardened the tree may be considered established. In every instance a normal tree was later formed, even though the initial flush might have been a weak one.

Relation of growth to temperature.

Girton (1927) determined that citrus seedlings grown in culture solutions under controlled conditions had an optimum root-growth temperature of around 78° F. and a zero or minimum at about 55° F. No evidence has been found as to the corresponding temperatures for top-growth ; it is reasonable to assume that such temperatures would be in the same range as for root-growth. Waynick and Walker (1930), Reed and MacDougal (1937), Cossman (1939), and Monselise (1944) all state that the most important ecological factor limiting citrus growth is low temperature.

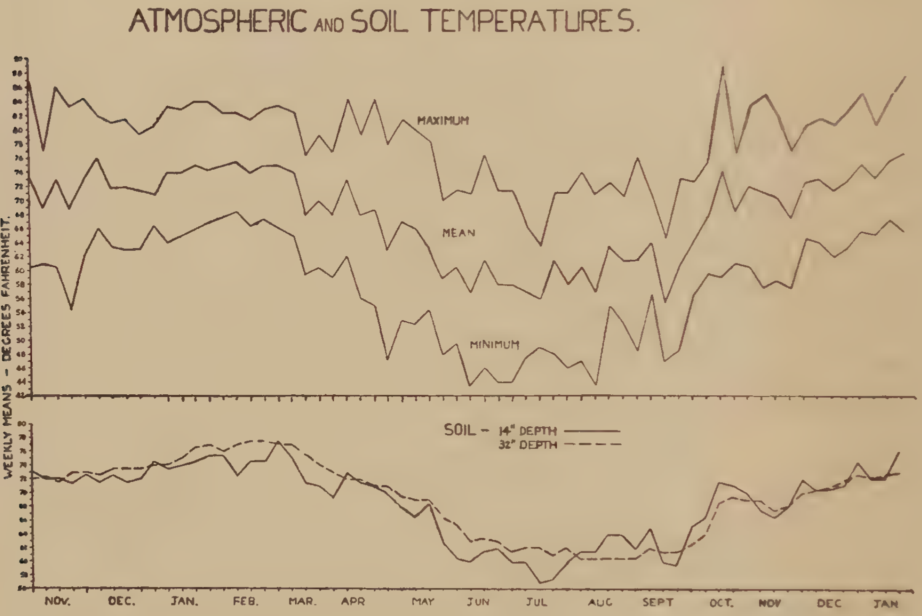

FIG. 3.

Mean weekly atmospheric and soil temperatures, November, 1938-January, 1940, Nelspruit, E. Tvl.

Fig. 3 presents the weekly means of the soil and atmospheric temperatures during the full period of growth observations. Soil temperatures were determined by four self-recording thermographs with the recording bulbs, two each at 14 inches and 32 inches depth, set in the soil amongst the roots at the drip of two trees. It is noted that the lowest mean weekly soil temperature was 57° F., the lowest absolute daily minimum having been 54° F. on two occasions. The lowest weekly mean of the daily mean atmospheric temperatures was 55·5° but, from May to September throughout, weekly mean minimum daily temperatures were below 55° but not lower than 43°.

From limited and incomplete observations Cossman (1939), working on bearing trees in Palestine, postulated that the factor limiting intensity of citrus root-growth in most winters is soil temperature, although the soil temperatures recorded were not lower than 59° F., and Monselise (1944) supports this view. With young citrus seedlings and budlings in a climate with mild winter and summer rainfall and of which the roots were never stressed for water, the author cannot find any specific correlation between soil temperatures above 57° F. and periodicity of root-growth. On the contrary, the most active period of growth was during the winter months.

If one accepts that the major spring top-growth flush is a direct effect of gradually rising atmospheric temperatures and not a cyclic expression of the physiological metabolism or life of the citrus tree, then such activity shows a definite relationship between temperature and shoot-growth. However, it can be seen by comparing Figs. 1 and 3 that soil temperatures are still near their lowest when this spring flush is at its height, and only thereafter do soil temperatures make an appreciable rise. It was noted that periodic low minimum atmospheric temperatures during winter brought about slight checks in shoot-growth, and resulted in more rapid hardening of new growth already made but the winter temperatures, both soil and atmospheric, had no direct effect on the very variable degree of minor flushing of the tops which occurred throughout the winter.

Control of periodicity.

It is conceded that in climatic areas where soil temperatures fall below 55° F. and mean daily atmospheric temperatures also are below this figure, soil temperature for root-growth and air temperature for top-growth may be the most important limiting factors. However, in view of the observations presented here, it is suggested that some internal factor or a combination of external factors working on the internal factor is the main controlling influence in the periodicity of root- and shoot-growth of young citrus trees when grown in climates with mild winters, especially in tropical climates where practically no seasonal fluctuation in temperature occurs.

The relationship between soil moisture and growth has not been dealt with here, as the variation in soil moisture for the plants worked with was not allowed to extend outside the limits of practical optimum. Waynick and Walker (1930), Cossman (1939), and Monselise (1944) all produce evidence that the lack of soil moisture or soil humidity is a distinct limiting factor to citrus root-growth. Conversely, excess soil water in the root zone, which would deprive the roots of aeration, would result in a checking of root-growth.

PRACTICAL APPLICATIONS.

The citrus growers' main interest in root-growth and top-growth of young citrus trees is concerned with the degree of success obtained in establishing a new orchard and in the rapid "getting-away" of the trees. Up to now, rough lemon has been the only stock used by commercial nurserymen in South Africa and, in general, no undue difficulties are met with in planting out trees successfully on this stock. Wide latitude exists in time of planting out, although the winter is usually avoided, particularly in areas experiencing frost, and a 2 per cent. loss in newly-planted orchards is accepted as normal.

From experience it can be stated that the following five factors are responsible for practically all losses of newly-planted trees: (1) Over dosage during fumigation after lifting from the nursery. (2) Absorption of HCN gas, during fumigation, by bare roots with free water on them through excess wetting. (3) Drying out of roots through exposure between lifting from the nursery and planting in the orchard.

(4) Planting in and covering roots with hot dry soil. (5) Inadequate watering in the root zone from immediate time of planting till trees have established themselves, which is after the first flush has hardened. Frosts, ants, sunburn, and mechanical injury account for the remaining losses.

An increasing use by growers of trees on sweet orange stock has shown that it is far more difficult to establish an orchard with trees on this stock than with the hardy rough lemon. Nurserymen and growers have been used to the hardiness of the rough lemon and thus do not give to trees on sweet orange the care they require. But even with care, as has been found in these experiments, time of planting-out is far more important with sweet orange than with rough lemon.

Fig. 2 supports practical experience that the most favourable time for nurserymen to transplant their *seedlings* from the seed beds into the nursery rows is during November. However, it is quite safe to do this planting-out with both stocks any time from the end of July to the end of March but, if it is done from the beginning of April to the end of July, difficulty will be experienced in obtaining satisfactory growth and far more after care will be needed.

In planting-out *budded trees* most rapid subsequent shooting was also obtained with those planted in November but the safe period, while also starting in July and lasting till the end of March for trees on rough lemon stock, was definitely curtailed for trees on sweet orange stock. This is a most important practical application, and growers planting trees on sweet stock are advised to do so in the most favourable period from mid-November to mid-January.

It is an accepted rule with nurserymen not to lift citrus trees from the nursery when they are in a flush, but to wait until the tops are hardened or dormant. In these experiments, trees lifted in all stages of top-growth throughout the year showed no correlation between their growth stage and the subsequent speed with which they produced new growth after planting, time of the year being the main determining factor. Actually, trees at the height of spring and summer flushes responded best.

Since it has been shown that root-growth is active in subtropical climates throughout the winter months, it is important that young trees be adequately supplied with soil moisture during this period, otherwise the root-growth will be checked, with subsequent retardation in rate of development of the tree. Thus, in summer rainfall areas, adequate winter irrigation should be practised.

SUMMARY.

1. This paper reports root-growth and top-growth observations on rough lemon and sweet orange seedlings, on Valencia budlings on these stocks, and on their subsequent shooting after being planted out at intervals throughout the year, under conditions of a summer rainfall and mild winter subtropical climate.

2. The general trend of root-growth and top-growth behaviour was the same for seedlings and budlings and also for rough lemon and sweet orange as seedlings or as stocks for budlings.

3. Seedlings exhibited a greater degree of activity in both root-growth and top-growth than did budlings, and rough lemon a greater degree than sweet orange either as a seedling or budling stock.

4. The periodicity of root-growth in citrus was confirmed. Two major periods and three minor periods were recorded ; the longer major period is during the winter from May to August with a second major period in mid-summer; a certain amount of indefiniteness is associated with the minor periods.

5. Although distinct cycles for major top-growth or flushes were exhibited, periodicity of top-growth in young seedlings and budlings was very variable. Four periods of top-growth were recorded, two definite and two indefinite, the major ones being consecutive, one in spring and the other in mid-summer.

6. The trend, in general, towards alternation in periodicity of root-growth and top-growth existed. Because of the distinct and sufficiently large variations to this alternation it is held to be doubtful if it can be accepted as a basic physiological characteristic of citrus.

7. On transplanting, seedlings established themselves more easily than did budlings, and rough lemon budlings much more easily than did budlings on sweet orange. The most favourable time for transplanting was November but rough lemon had a wide safe range from August to March while the period for sweet orange was much shorter, being from November to January.

8. While, in colder climates, the main factor limiting citrus root-growth, either directly or indirectly, is accepted to be soil temperatures, no specific correlation between soil temperatures above 57° F. and periodicity of root-growth was found with young plants. Top-growth did not show any definite correlation with soil or atmospheric temperatures.

9. It is suggested that the control of periodicity of root-growth and top-growth is determined by an internal factor such as hormonic production and communication, or by the influence of ecological factors thereon, rather than by a direct effect on the growing points themselves.

10. Among the practical applications of this work discussed are the most favourable times for nurserymen and growers to plant out nurseries and orchards in order to obtain best subsequent growth, the factors which cause losses on such planting-out, and the necessity for winter irrigation in summer rainfall areas.

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THE MANGANESE AND IRON CONTENTS OF CROP PLANTS AS DETERMINED BY CHEMICAL METHODS

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SYNOPSIS

Rapid chemical tissue tests for manganese are described which can be used for the determination of the element at deficiency and toxicity levels in plant tissues. The method has been used for the study of the manganese status of a wide range of crop plants growing at centres differing markedly in soil characters.

It is shown that the manganese contents of plants vary considerably with the soil pH whereas the iron levels in the tissues are relatively constant irrespective of soil acidity or alkalinity. The ratio of " total " iron to manganese in plant tissues does not appear to be of importance in the diagnosis of a deficiency or toxicity of manganese in the crops studied. The availability of manganese to the various crops in the three types of soil was reflected by the manganese levels found in the plants.

It has been shown that by using a tissue test method it is possible to determine Mn at deficiency levels in cereal crops (19, 20). In the present investigation the applicability of this sensitive test for Mn to other crops has been examined as well as the relation between tissue test and ash analysis data for the element.

In addition, the total Fe and Mn contents of a variety of crop plants have been examined to determine whether the Fe/Mn ratio is a crucial factor in the production of deficiencies of Mn and Fe in crops as has been suggested from the work of Somers *et al.* (24). These workers showed that there is a marked antagonism between the two micro-nutrients ; in their experiments, Mn deficiency was produced in Soya bean grown in water culture by high levels of Fe in the nutrient solution and *vice versa.* Their results indicated that for optimum growth the Fe/Mn ratio in the nutrient solution must approximate to 2. Using water culture methods, Bennett (5), Chapman *et al.* (7), Pearse (21), Schapelle (23), and Twyman (25) have confirmed the existence of a relation between the two micro-nutrients although there is a difference between the suggested actual optimum ratios.

The importance of the Fe/Mn ratio in practice has been shown in studies of manganiferous soils in Puerto Rico (15) and Hawaii (17) where the high Mn levels induce iron deficiency chlorosis in pineapple.

The work described in the present paper was carried out at three centres, providing different levels of Mn in the soils, at which several crops showing a considerable range of susceptibility to Mn deficiency were grown. At one centre many crops showed marked symptoms of manganese deficiency ; at the second manganese status was also low but only highly susceptible crops showed evidence of deficiency ; at the third, on a strongly acid soil, manganese level was high and potatoes showed signs of Mn toxicity. The crops grown were selected to include a number susceptible to both Mn and Fe deficiencies, e.g. potato (26). The experiments were continued for two seasons.

The results show that the Mn deficiency and toxicity effects observed in the plants can be explained on the basis of the Mn contents of the plants. The Fe contents of plants did not appear to be a critical factor in determining Mn deficiency as the range of Fe found was relatively small. Moreover, although very high values for manganese and low Fe/Mn ratios were obtained at the centre where Mn toxicity occurred, iron deficiency was not recorded.

EXPERIMENTAL.

Three centres were used and details of location, soil characters, crops grown and the outstanding nutritional problems occurring at each centre are given in Table I.

The following supplementary notes are given for each centre.

Centre A. The soil is a sandy loam derived from Pennant Grit, in an area previously used for market garden crops and now a well cultivated private garden.

TABLE I.

Data relating to soil characters, crops grown and the nutritional problems at Centres A, B and C.

Centre.	Soil characters.	Crop and Variety.	Nutritional Problems.
A. Old Garden	Sandy loam, derived from Pennant Grit of the Coal Measures Formation.	*Runner Bean :* Scarlet Emperor. *Dwarf Bean :* Masterpiece, Lightning, Comtesse de Chamborde, Foremost. *Broad Bean :* Triple White, Long Pod, Great Windsor. *Tick Bean.* *Pea :* Onward, Foremost, Kelvedon Wonder. *Potato :* Pilot, Majestic. *Turnip :* Green Top Stone. *Mangold :* Golden Tankard. *Sugar Beet :* Selected Strain. *Swede :* Purple Top. *Kale :* Marrow Stem, Hungry Gap. *Lettuce :* Cos, Cabbage. *Rape :* Black. *Flax :* Liral Prince. *Wheat :* Red Marvel. *Barley :* Plumage Archer. *Oat :* White Star. *Maize :* Golden Bantam. *Clover :* Kent White, Dorset Marvel. *Lucerne.* *Radish :* Sparkler.	Mn deficiency.
B. Long Ashton. Long term manurial trial (17A).	Calcareous sandy loam, derived from red drift overlying Keuper Marl.	*Wheat :* Red Marvel. *Barley :* Spratt's Archer. *Potato :* Majestic, Kerr's Pink. *Sugar Beet :* Selected Strain. *Mangold :* New Cross. *Cauliflower :* Majestic.	N, P, K, Mg, P and Mn deficiencies in susceptible crops.
C. Sutton Park Birmingham.	Highly podsolised gravelly sand, mainly derived from glacial deposits of Bunter origin.	*Potato :* Majestic.	N, P, K, Mg and Ca deficiencies and Mn and Al toxicities in susceptible crops.

It is a typical old "black" garden soil, previously heavily manured with stable manure and regularly limed. Many vegetable and fruit crops show well marked symptoms of Mn deficiency and highly susceptible crops, e.g. oat, grown experimentally, have failed completely. Relevant analytical results for this soil (pH 7·1) are as follows :—

Organic carbon	6·5%
Free $CaCO_3$	present.
Exchangeable K_2O	0·039%
Exchangeable P_2O_5	0·234%
Water-soluble Mn	Nil.
Exchangeable Mn	1·1 p.p.m.
Easily reducible Mn	31 p.p.m.
Total Mn	341 p.p.m.

Centre B. Use was made of a long term manurial trial in which six treatments are represented : Nil ; stable manure, F.Y.M. ; complete fertilizer, NPK ; omit nitrogen, PK ; omit phosphorus, NK ; omit potassium, NP.

The annual rates of application are as follows :—

F.Y.M.	10 tons per acre.
Nitrogen	4 cwt. of nitro-chalk per acre.
Phosphorus	3 cwt. of superphosphate per acre.
Potassium	2 cwt. of sulphate of potash per acre.

The treatments occur in triplicate in blocks, magnesium sulphate at 1 cwt. per acre being applied annually to one block (block II).

The surface soil and subsoil contain approximately 5 and 1 per cent. free lime respectively with a pH ranging from 7·2 to 7·4 in the top soil. The exchangeable Mn and easily reducible Mn were 6·5 and 48 p.p.m. respectively. Mn deficiency was recorded only as a trace in a few crops, e.g. sugar beet and mangold, early in the growing season, and is not of economic importance.

Centre C. The experimental area is a reclaimed acid heathland (originally with a soil of approximately pH 4·0). It is divided into strips to which different rates of ground limestone were applied in 1944 and across which various fertilizer treatments, consisting of various NPK combinations, are given annually. The rates of application of ground limestone and NPK fertilizers are as follows :

Ground limestone at 3 and 9 tons per acre respectively.
Nitrogen (N), as sulphate of ammonia, at 3 cwt. per acre.
Phosphorus (P), as triple superphosphate, at 2½ cwt. per acre.
Potash (K), as muriate of potash, at 2½ cwt. per acre.

By contrast with centres A and B, at this centre susceptible crops, viz. potato, show visual signs of Mn excess and, in addition, Ca, K, Mg, P and N may be deficient (29).

METHODS FOR CHEMICAL TISSUE TESTS AND ASH ANALYSIS.

Selection of plant parts.

Details of the field sampling technique have been given in previous papers (18, 19, 20, 22). The following crops, not previously mentioned, were sampled as follows :

Bean, pea, lucerne ..	Leaves from the mid-stem portion of the plants.
Rye and maize ..	Internode adjacent to the growing point.
Flax	Stem portions midway between mid-stem and growing point.
Kale, rape	First upright leaf outside incurled leaves.
Clover, radish, carrot	Leaves located midway between young and old foliage.

Preparation of test samples.

The material was cleaned with a muslin cloth to remove small particles of dust adhering to the outer surfaces.

(*a*) *Tissue tests.* When using broad-leaved crops, small petiole portions, half way between leaf attachment and the base of the lamina, were taken. For the Brassicae, mid-rib portions of comparable girth, near the tip of the lamina were used. In the cereals the stalk portion was generally used, although at the very young growth stage the first-formed leaf was sometimes taken.

(*b*) *Ash analysis.* The leaves, including the petiole, were dried for 48 hours in a specially aerated oven and then, while still warm, were crushed by hand, using rubber gloves. The dry material was stored in inert plastic bags.

A wet digestion procedure used for the removal of organic matter has been previously described (20). Approximately 5 gm. of dry matter was placed in a 400 ml. Pyrex beaker fitted with a watchglass and 15 ml. of HNO_3 (conc.) per gm. of dry matter, added. After heating on an Argand burner for approximately 30 minutes to remove ammonia, the digest was cooled and 1 ml. of $HClO_4$ per gm. of dry matter was added slowly and heating continued until the " frothing " stage when oxidation of organic matter takes place. At this stage the oxidation process was continued at a lower temperature but more vigorously later until the silica had been dehydrated. Hot glass-distilled water was added and, after heating on a hot plate at 80° C. for 30 minutes, the solution was cooled and filtered through a No. 42 Whatman paper into a 100 ml. graduated flask.

Extraction of soluble manganese.

Morgan's reagent, consisting of 100 gm. of sodium acetate and 30 ml. of glacial acetic acid at pH 4·8, was used for the extraction of soluble Mn. The reagents had to be specially purified for the extraction of Mn from healthy and Mn deficient plants (20) (Centres A and B).

The quantities recommended in previous papers (18, 22) were used, viz., 1 gm. of finely chopped tissue placed in a specimen tube with 10 ml. of Morgan's reagent. After 15 minutes, 5 ml. of the leachate was pipetted into a clean specimen tube and the test for Mn made on the 5 ml. extract.

Chemical tests used.

Tissue tests. Tetramethyldiaminodiphenylmethane was used for the detection of normal and deficiency levels of Mn in tissue extracts (20). The tetrabase was recrystallized before use, using redistilled methyl alcohol. The tetrabase should be colourless when dissolved in acetone.

A series of colour standards, using bromothymol blue, thymol blue, bromophenol blue and dilute ammonia, were employed in the determination of Mn by the tetrabase method (20).

The test is carried out by adding 2 ml. of a saturated solution of KIO_4 to a tissue extract chilled in ice, followed by 0·2 ml. glacial acetic acid (redistilled) and 0·4 ml. of 1 per cent. tetrabase in redistilled acetone. In legumes, it was found necessary to add 5 ml. of KIO_4 because of the presence of reducing compounds which interfere with the test. When KIO_4 was added to tissue extracts of legumes, e.g. broad bean and runner bean, iodine was liberated and this precluded the use of the test. Heat treatment and the use of a number of oxidizing reagents failed to remove the interfering substances but the addition of 5 ml. of KIO_4, followed immediately by 0·4 ml. of tetrabase to the extract previously cooled for 5 minutes in ice, produced the true blue Mn colour. Further work is necessary to determine the factors responsible for this interference. The stability of the colour was improved by cooling the tissue extract in ice before testing.

The formaldoxime test (18, 22) was used to determine Mn when present in excess in tissue extracts. To the 5 ml. extract was added 0·5 ml. of formaldoxime and 2 ml. of 10 per cent. NaOH. The cherry brown colour produced was matched with a series of prepared standards made up in Morgan's reagent covering the range 0·5, 1, 2 to 10 p.p.m. Mn. A suitable calibration diagram was prepared, using an absorptiometer.

Ash analysis. (1) *Manganese.* In the material from healthy and Mn deficient plants, 90 ml. of the ash solution was evaporated to dryness in a silica basin. After cooling, 5 ml. of conc. HNO_3 and 20 ml. of glass-distilled water were added and the solution heated for 15 minutes at 60° C. so that the nitrates dissolved. The solution was then filtered through a No. 41 Whatman paper into a conical flask, 5 ml. of phosphoric acid was added, followed by 0·2 gm. of sodium periodate and the solution was heated on the hot plate. The potassium permanganate so formed was made up to 100 ml. with 2 N. H_2SO_4 previously boiled with KIO_4 and determined by means of the Spekker Absorptiometer with a water setting of 1 and using 1 cm. cells and Ilford green filters. The effective range is from 0·002 to 0·05 mg. Mn per 100 ml. When Mn was present at very low levels it was necessary to wet digest two separate lots of 5 gm. dry matter and these were bulked for the determination. When Mn was present in excess 25 ml. of the 100 ml. stock solution was sufficient.

(2) *Iron.* The familiar ammonium thiocyanate method was used for the determination of iron. The ferric thiocyanate was extracted with 10 ml. of a 1 : 1 mixture of amyl alcohol and amyl acetate. The solvent layer was pipetted into a centrifuge tube and spun for 5 minutes at 2,500 revs. per minute to clear. The Fe was determined by means of the Spekker Absorptiometer using blue-green Ilford filters, a 1 cm. cell and a water setting of 1. The effective range is from 0·005 to 0·04 mg. per 10 ml.

RESULTS

Centre A.

During 1946 and 1947, the easily soluble Mn and total Mn and Fe were determined in a number of crop plants at fortnightly intervals, making a total of seven determinations. The data for 1946 were similar to those of 1947 and for this reason the data for 1947 only are shown. The data in Table II include the mean values

TABLE II.

Centre A. Mean values for Mn and Fe in crop plants for season 1947, based on seven determinations during the growth period.

Crop.	Tissue Test. Mn.	Ash analysis		Fe/Mn Ratio.	Symptoms of Mn deficiency.
		Mn.	Fe.		
RUNNER BEAN: Prince	12	13	197	15	Trace.
DWARF BEAN: Masterpiece	13	15	148	10	Nil.
Light	10	12	240	20	Trace.
Comtesse de Chamborde	11	13	206	16	Nil.
Foremost	14	16	229	14	Nil.
BROAD BEAN: Triple White	10	11	183	17	Trace.
Longpod	10	10	214	21	Medium.
Great Windsor	12	14	170	12	Trace.
TICK BEAN	11	13	258	20	Trace.
PEA: Onward	9	10	130	13	Trace.
Foremost	8	8	234	29	Medium—Pronounced.
Kelvedon Wonder	9	8	219	27	Medium.
POTATO: Pilot	12	13	203	16	Nil.
Majestic	10	11	224	20	Trace.
TURNIP	9	11	156	14	Trace.
MANGOLD	9	10	145	15	Medium.
SUGAR BEET	9	10	189	19	Medium.
SWEDE	10	14	191	14	Nil.
KALE: Marrow Stem	12	12	135	11	Nil.
Hungry Gap	12	14	109	8	Nil.
RAPE	8	11	160	15	Nil.
LETTUCE: Cos	6	8	258	32	Pronounced.
Cabbage	16	18	226	13	Trace.
FLAX	16	18	237	12	Nil.
WHEAT	14	14	197	14	Trace.
BARLEY	13	12	184	15	Trace.
OAT	7	7	162	23	Pronounced.
MAIZE	12	15	196	13	Nil.
RADISH	15	19	77	4	Nil.
CLOVER: White	14	14	196	14	Nil.
Red	10	12	125	10	Nil.
LUCERNE	15	16	151	9	Nil.
CARROT	11	16	160	10	Nil.
CAULIFLOWER	11	10	115	12	Trace.

for seven determinations for soluble Mn and total Mn and Fe, the mean Fe/Mn ratios and a record of Mn deficiency symptoms.

The main observations are as follows: The Mn levels, whether determined by tissue tests or ash analysis, are low throughout. Flax, lucerne, carrot, maize and radish are among those with 15 p.p.m. Mn or over and, in these, visual signs of the deficiency were absent whereas in oat, sugar beet, mangold, cos lettuce and pea (variety Foremost) the values are 10 p.p.m. or under and these crops showed signs of Mn deficiency. Interesting varietal differences are also shown; Cos lettuce has a lower level than cabbage lettuce and the former was more susceptible to the deficiency. The variety Foremost was more susceptible to the deficiency than were the other two varieties of pea. Amongst the cereals, oat, with 7 p.p.m. is the most susceptible, whereas maize with 15 p.p.m. is the least.

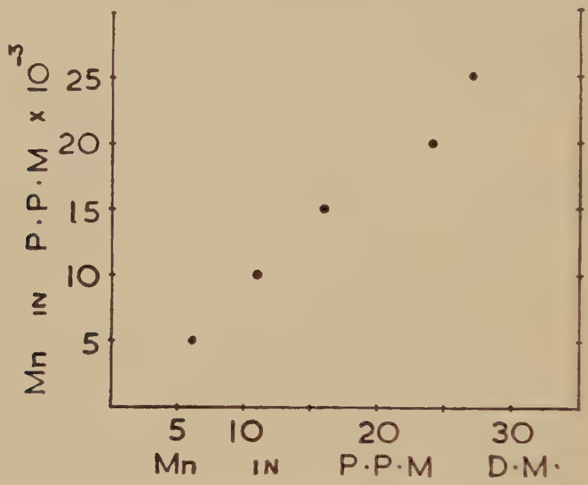


FIG. 1.

The relation between tissue tests (ordinates) and ash analyses (abscissae) for Mn in all crops at Centre A. Tissue test and ash analysis data given as p.p.m. × 10^{-3} in the extract and in p.p.m. in dry matter respectively. The means of the total Mn values per tissue test category are plotted.

Low Mn values were not always associated with deficiency symptoms and it appears that despite low levels some crops can function without obvious deleterious effects.

The Fe levels appear to be adequate throughout as no visual signs of the deficiency appeared in any of the 34 crops studied. The Fe/Mn ratios given in Table II show that these are usually high in crops susceptible to Mn deficiency, viz. oat, 23, as compared with maize at 13; lettuce (Cos), 32, whereas the cabbage variety is 13; pea, varieties Foremost and Kelvedon, 29 and 27 respectively, as compared with the variety Onward at 13.

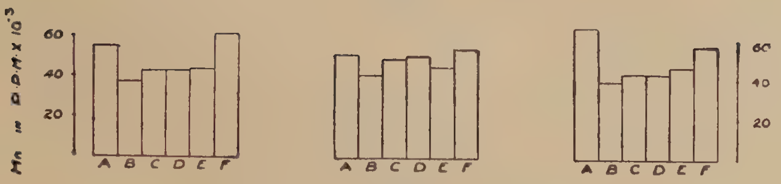
Fig. 1 shows the relationship between the tissue test and ash analysis data. The graph was constructed from 290 tissue test and total manganese values for 34 crop plants. In view of the wide differences in Mn content of the different plants, the graphs are constructed from mean values of total manganese

FIG. 2.

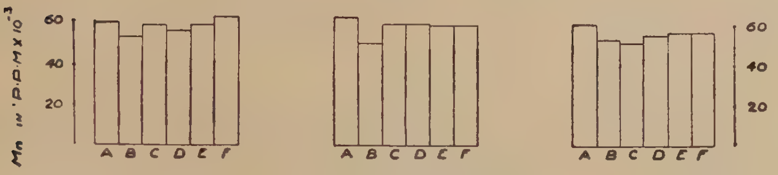
Season 1946.

MANGANESE

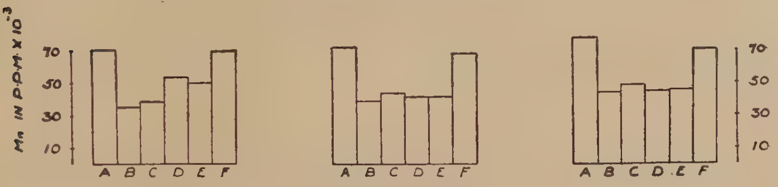
Wheat.



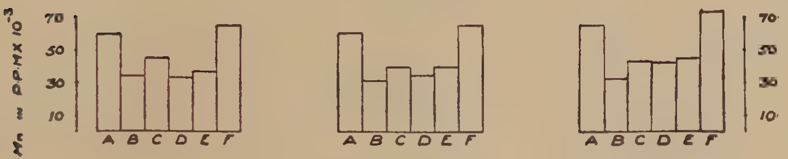
Barley.



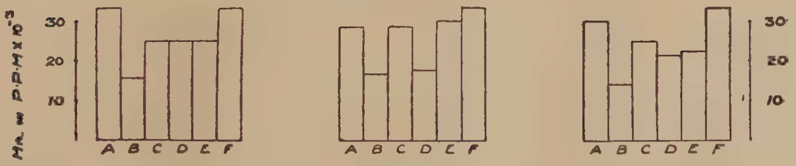
Potato (Kerr's Pink).



Potato (Majestic).



Sugar Beet.



Mangold.

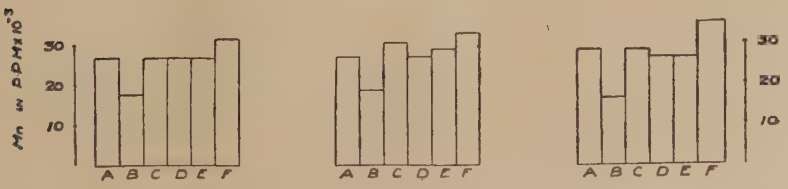
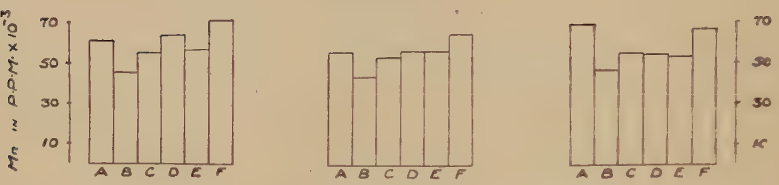


FIG. 2—*Continued.*

Cauliflower.



Histograms representing the mean of the six seasonal tissue test values for Mn in crops growing under various manurial treatments, viz. :

A=Nil ; B=Stable manure; C=NPK ; D=PK (omit N); E=NK (omit P); F=NP (omit K).

The three histograms in each set refer to the three blocks in the experiment.

for each tissue test category. The relation shown is linear, thus demonstrating the value of the tissue test method as an indicator of manganese level in the plant.

The seasonal cycles of the total Mn and Fe contents of the crops were also examined but they did not show any definite trends during the two seasons. The Mn levels were low in most crops studied, at all seven sampling dates, and reflected the low availability of Mn in this soil.

Centre B.

At this centre, Mn was determined by tissue test and ash analysis at intervals during the 1946 season in wheat, barley, potato (varieties Majestic and Kerr's Pink), cauliflower, sugar beet and mangold. Six sets of tissue tests were made at fortnightly periods and ash analysis of samples three times at monthly intervals.

Tissue tests in relation to manurial treatment. Histograms based on the mean tissue test values for each treatment for the season 1946 are given in Fig. 2.

The results show that the values are higher than for corresponding crops at centre A and this agrees with the absence of visual symptoms in all crops other than sugar beet and mangold. Although the Mn values for the last-named crops are the lowest for this centre, they are higher than those recorded at centre A. In potato and sugar beet the nil and NP (omit K) plants usually have a higher Mn level whereas those supplied with stable manure have a lower mean than have those under other treatments. In the stable manure treatment the low Mn value probably reflects a reduction in the availability of Mn resulting from the addition of organic matter to a calcareous soil (16).

Seasonal cycle of Manganese in crops. The seasonal cycles of soluble Mn in NPK, F.Y.M. (stable manure) and NP (omit K) treatments are given in Fig. 3.

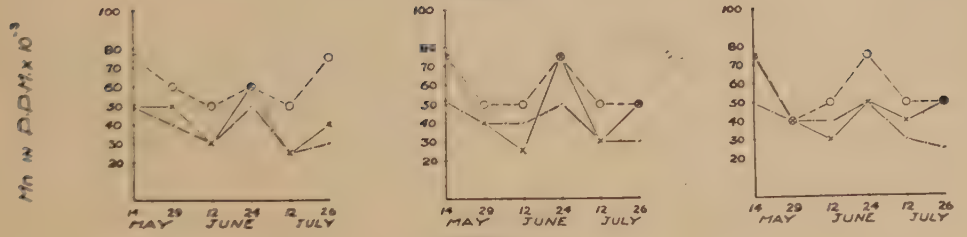
In wheat and barley there is no definite seasonal trend. In potato and cauliflower there is a tendency for values to fall throughout with advance of season, whilst in sugar beet and mangold, values for all treatments are low in the early season and increase from mid to late season. In the root crops the cross-hatched band between 10 and 20 × 10^{-3} p.p.m. Mn coincides with the onset of visual symptoms of the deficiency. After 25th July and 7th August, in mangold and sugar beet

FIG. 3.

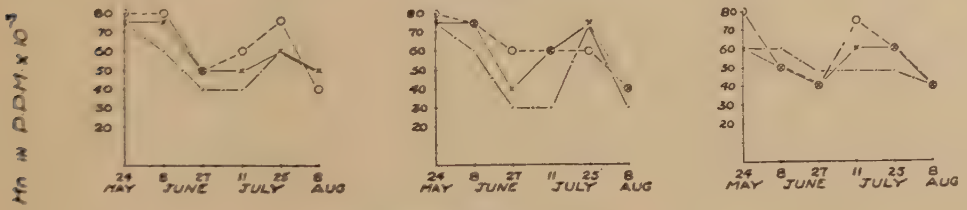
Season 1946.

MANGANESE

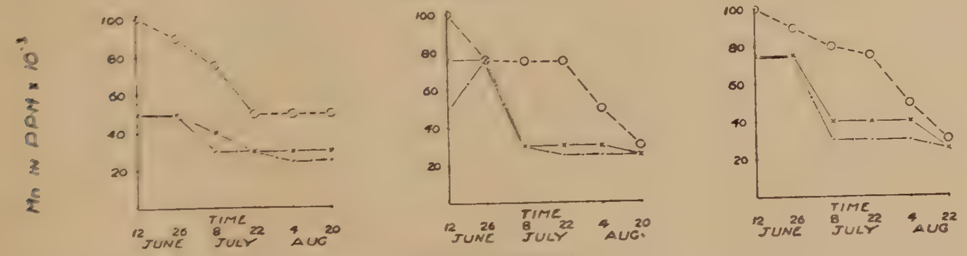
Wheat.



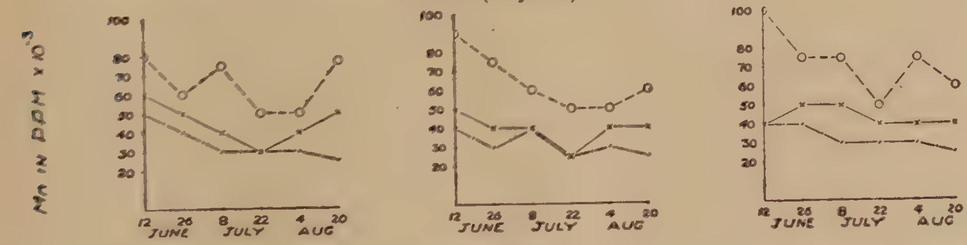
Barley.



Potato (Kerr's Pink).



Potato (Majestic).



Sugar Beet.

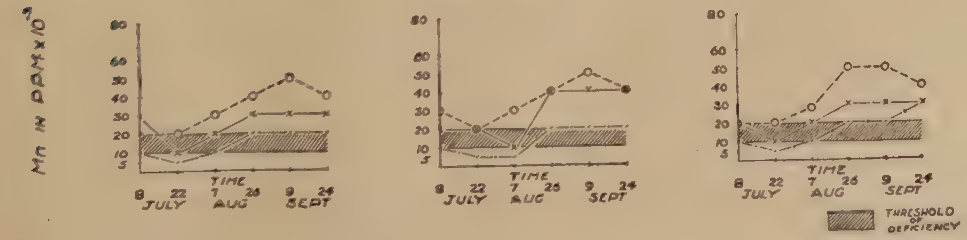
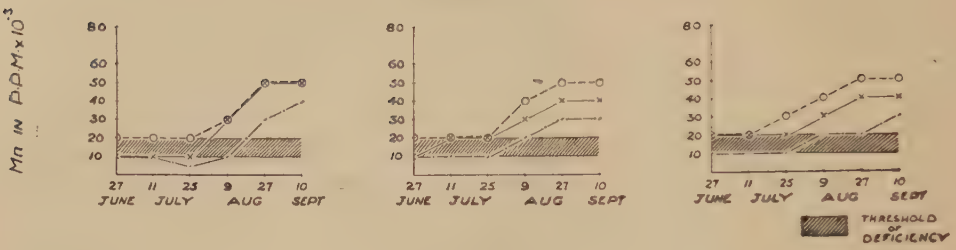
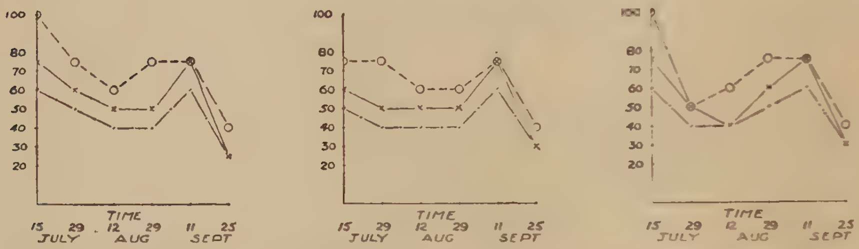


FIG. 3—*Continued.*

Mangold.



Cauliflower.



The seasonal tissue test values for Mn in Wheat, Barley, Potato (Kerr's Pink and Majestic), Sugar Beet, Mangold and Cauliflower.

Treatments: X———X NPK ; O — — O NP (omit K) ; I—— · ——I Stable manure.

The three graphs in each set refer to the three blocks in the experiment.

respectively, there is a general increase irrespective of treatment and visual signs disappear. The NP (omit K), NPK, and stable manure treatments recover in that order.

Relation between tissue tests and ash analysis. Comparisons between the results of the two chemical methods were made at intervals during the 1946 and 1947 seasons. Fig. 4 shows the relation between tissue test results (as p.p.m. × 10^{-3} in the extract) and ash analysis data for Mn (as p.p.m.) dry matter. For each crop plant the tissue test results for Mn are plotted, irrespective of treatment, as ordinates and ash analysis data as abscissae. The means of the total Mn values per tissue test category, as in Fig. 1, are again used. By plotting the results for the three seasonal sampling times, making a total of 54 determinations, a relationship curve is obtained for each crop. The agreement between the two methods is close, indicating the suitability of the tissue test procedure to reflect the Mn status of the plants.

Centre C.

The Mn, Fe and Ca contents of the foliage of potato grown at this centre were estimated by ash analysis and the extractable Mn was determined by a tissue test method, using the formaldoxime reagent to detect the micro-nutrient at high levels (29). The data for seasons 1946 and 1947 are given in Table III and the relation between total Mn, Fe and Ca under various NPK and liming treatments is shown in Fig. 5.

The Mn levels are high in the unlimed plants whether determined by tissue test or ash analysis and values for Ca are low. In the limed samples the Ca levels are high and Mn values correspondingly low. The results for the tissue test and ash analysis for both series show good agreement.

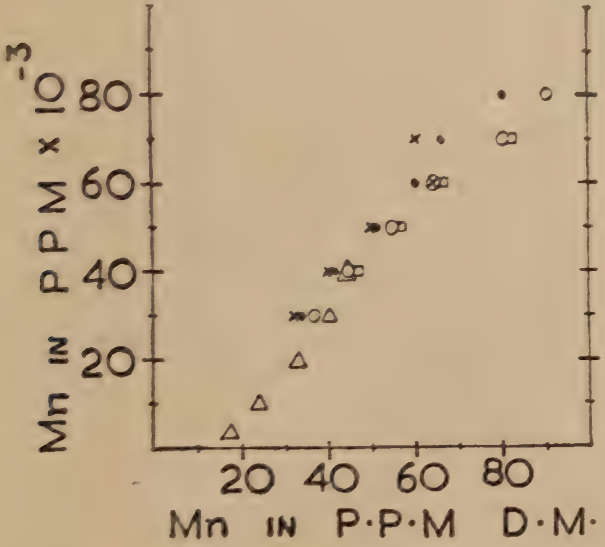


FIG. 4.

The relation between tissue tests (ordinates) and ash analysis for Mn in crop plants. Tissue test and ash analysis data as p.p.m. × 10^{-3} and in p.p.m. dry matter respectively. The means of the total Mn values per tissue test category are plotted.

● **WHEAT.** □ **CAULIFLOWER.** △ **SUGAR BEET.**
X **BARLEY.** O **POTATO (Majestic).**

Visual signs of Mn toxicity (12, 13, 18, 26, 27, 29) were recorded in the unlimed plots but were absent from the limed group. Symptoms of K, Mg, Ca, P and N deficiencies were observed, corresponding with manurial treatments. The Fe content of the leaves is lower than for potato grown at centre A, where Mn was deficient but there is no significant difference between the Fe status of the limed and unlimed plants as recorded for Mn. The Fe contents appeared adequate throughout and visual signs of Fe deficiency were not observed although potato may be regarded as a crop liable to show Fe deficiency (26).

TABLE III.

Visual symptoms, tissue test and ash analysis data for potato (Majestic) for seasons 1946 and 1947.

Plot No.	Manurial Treatment.	Visual Symptoms (−deficiency + toxicity).		TISSUE TEST.				ASH ANALYSIS.						Fe/Mn Ratio.	
				(as p.p.m. in extract).				as % dry matter		as p.p.m. in dry matter.					
				Ca.		Mn.		CaO		Mn.		Fe.			
		1946.	1947.	1946.	1947.	1946.	1947.	1946.	1947.	1946.	1947.	1946.	1947.	1946.	1947.
	No lime plots.														
1	Nil.	−Ca	−P	25	25	5	8	0·70	1·09	390	422	100	49	0·27	0·12
2	Nil.	−K+Mn	+Mn	25	60	10	8	0·80	0·79	639	335	97	42	0·14	0·13
3	N P K	−Ca−Mg+Mn	−Ca	25	25	10	8	0·82	0·83	540	536	80	76	0·15	0·14
4	P K	−Ca−Mg+Mn	−Ca	25	25	5	8	0·54	0·64	457	481	76	73	0·17	0·16
5	N K	−Ca+Mn	−P−Ca	25	25	5	8	0·78	0·86	303	333	85	48	0·25	0·14
6	K	−Ca−Mg+Mn	−P	25	25	5	8	0·63	0·73	326	367	106	53	0·33	0·14
7	N P	−K+Mn	−K−Ca+Mn	60	25	10	10	0·96	0·61	485	300	98	42	0·20	0·14
8	P	−K+Mn	−Ca+Mn	60	60	10	10	1·0	0·56	620	394	78	50	0·13	0·13
	Plots limed at 9 tons/acre.														
9	Nil.	−Mg	−P	95	130	0·8	1·0	4·01	4·59	130	108	76	88	0·60	0·81
10	Nil.	−K	−P	165	200	0·5	0·8	5·42	4·88	162	78	82	41	0·56	0·52
11	N P K	−Mg	−Mg.	130	130	0·8	2·0	4·52	4·35	141	119	75	70	0·53	0·59
12	P K	−Mg	−N−Mg	95	165	0·8	2·0	3·62	4·59	128	136	63	108	0·49	0·79
13	N K	−Mg	−P	130	130	0·8	2·0	3·92	3·37	117	91	84	48	0·71	0·53
14	K	−Mg−P	−P−Mg	130	95	0·8	0·8	4·06	4·11	136	75	96	66	0·70	0·88
15	N P	−K −P	−K	165	165	0·5	0·8	5·02	4·77	152	59	92	64	0·60	1·08
16	P	−K	−K	165	165	0·5	0·8	5·36	4·98	156	68	65	71	0·42	1·04
		−K													

The Fe/Mn ratio in the unlimed plots is very low, viz. 0·12 to 0·33 and in the limed plots is only 0·42 to 1·08. The lower range of ratios was associated with Mn toxicity but not with iron deficiency. The Mn values, as at the previous centres, were a better index of the Mn status of the plants than were the Fe/Mn ratios.

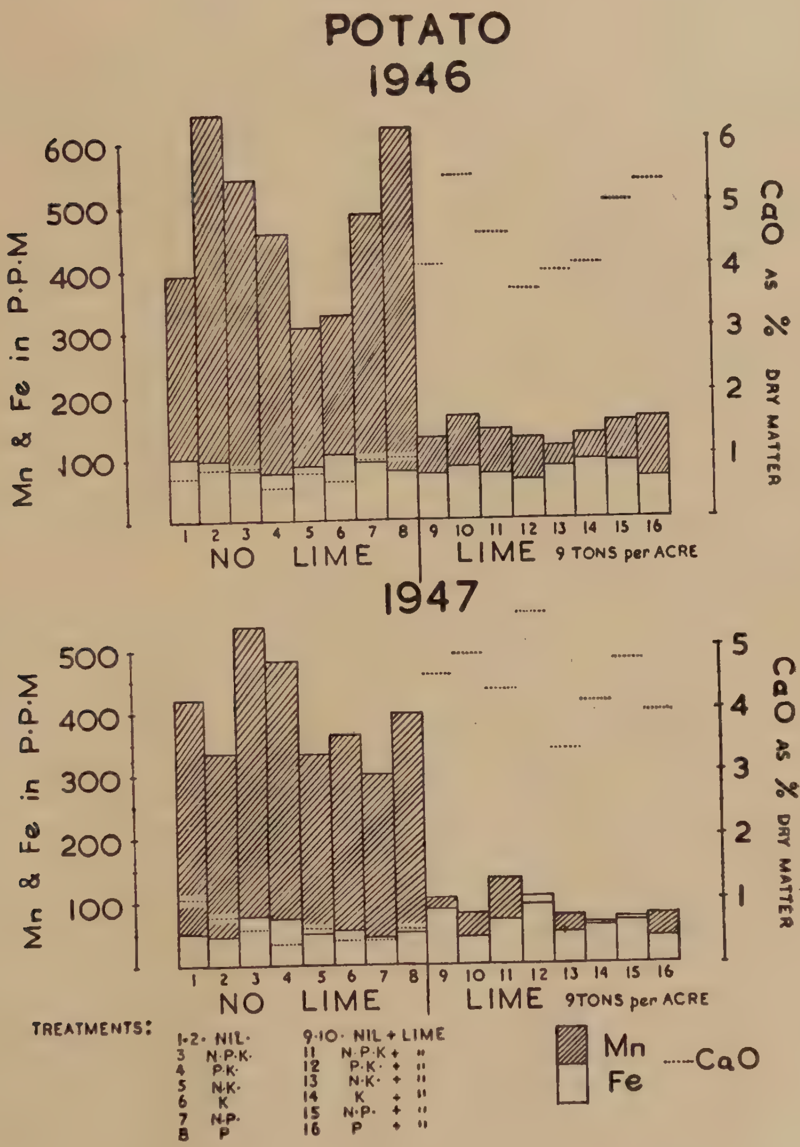


FIG. 5.

The Mn, Fe and Ca contents of Potato, variety Majestic, grown under various manurial treatments.

DISCUSSION.

The objects of these experiments were (*a*) to examine the applicability of tissue test methods in the diagnosis of the Mn status of a range of crop plants and (*b*), to determine whether the Fe/Mn ratio within the plants was a critical factor in the diagnosis of deficiencies or excesses of the two micro-nutrients.

At centres A and B, where Mn was very deficient and moderately low respectively, the sensitive tissue test for Mn, applied at intervals during seasons 1946 and 1947 to a variety of crop plants, showed good agreement with results of ash analysis. Similarly at centre C, where the Mn status reached toxic levels in potato in the unlimed plots, ash analysis confirmed the tissue test values. Thus the tissue test procedure for either deficiency or toxicity levels is valuable for diagnosis and because of its speed of application is preferable to the longer method of ash analysis.

At centre A, Mn values were consistently low throughout the season in the 34 crops studied. At centre B, where Mn was moderately low, wheat and barley showed no consistent trend, whereas values in potato fell with seasonal advance and those in root crops increased from deficiency levels at the beginning of the season to more normal values later.

The manurial effects on the Mn status in crops noted at centre B showed that Mn was usually higher when K was omitted and lower in the presence of stable manure. It appears that K acts as a protective against the intake of Mn, as does Ca ; this K effect was also noted at centre C where Mn values were usually higher when K as well as Ca were low in the plants. The lower Mn status in plants given stable manure may be due to a biological fixation of Mn in the presence of added organic matter in a calcareous soil (16).

Differences in susceptibility of crops to Mn deficiency were noted, e.g. oat was the most susceptible in the cereal group, Cos lettuce more so than the cabbage variety ; of the pea varieties, Foremost and Kelvedon were more susceptible than Onward. Flax, carrot, maize and clover appeared relatively resistant. Chemical data showed that plants susceptible to the deficiency usually had lower Mn values than those free from symptoms although values were very low in all crops at centre A and it is clear that susceptibility is linked not only with low chemical values but also with the ability of the plants to carry on their metabolism despite low levels.

At the three centres the Mn status of the plants provided a reliable index of the availability of Mn in the soil. This was shown by a positive correlation between severity of symptoms of either Mn deficiency or toxicity with the Mn levels in the plants. The Fe/Mn ratio in the plants varied from 32 to 4 at Centre A, to 1·08 to 0·12 at Centre C, where Mn deficiency and toxicity symptoms respectively were recorded but symptoms of iron deficiency were not observed. It appears that the Fe/Mn ratio can vary over a wide range without visibly affecting the iron metabolism of a variety of crop plants including some known to be susceptible to Fe deficiency. Moreover, studies in soil acidity have confirmed that although deficiencies of Ca, K, Mg, P, N (27, 29) and Mo (1, 2, 8, 9, 10, 14) and toxicity levels in Mn and Al (11, 12, 13, 27, 29, 30, 31) may occur in crop plants growing in acid soils, iron deficiency is seldom recorded. It has also been shown that symptoms of Mn excess may be quite distinct from those of iron deficiency and *vice versa* (12, 18, 28). The Fe/Mn relationships established by water culture experiments (5, 7, 21, 23, 24, 25) and confirmed in manganiferous soils at Hawaii and Puerto Rico by McGeorge (17) and Hopkins *et al.* (15) respectively, do not appear to be of major importance in this country for the diagnosis of Mn deficiency or toxicity in the field crops studied.

SUMMARY.

1. Three centres differing widely in soil characters were chosen to study the application of tissue test methods in the diagnosis of the Mn status of a variety of crops. The centres provided three levels of Mn supply ; one centre had a low level and a number of crops suffered from Mn deficiency; at the second, Mn was moderately low but sufficient for most crops ; and at the third, on an acid soil, the potato crop suffered from excess of Mn.

2. Good agreement was obtained between the results of tissue tests and those of ash analysis at all three levels of Mn and the quick methods thus appear useful for determining the Mn status of crops.

3. Crops and varieties particularly susceptible to Mn deficiency usually showed lower Mn values than did more resistant ones but some of the latter also gave low values although apparently they were able to grow fairly normally at low levels of the micro-nutrient.

4. There was no distinct seasonal trend for Mn in crops at Centre A, where the element was acutely deficient and the levels consistently low. At Centre B where Mn supply was only moderately low, distinct seasonal trends were recorded in Mn level for some crops, notably sugar beet, mangold, potato and cauliflower. In sugar beet and mangold the values rose from mid to late season and this was accompanied by the disappearance of Mn deficiency symptoms from these crops.

5. At Centre B the Mn contents of the various crops were affected by the different manurial treatments. In particular, high levels were found in NP (omit K) plants and low levels in those given stable manure. The latter effect may be due to increased biological fixation of Mn activated by the added organic matter.

6. At Centre C, in the acid soil, the potato crop under differential manuring showed symptoms of Ca, K, Mg, P and N deficiencies and of Mn excess. The symptoms of Mn toxicity were distinct from those of Fe deficiency and *Fe deficiency* was not recorded.

7. On the Mn deficient soil at Centre A the Fe/Mn ratios in the plants varied from 32 to 4, whereas at Centre C where Mn was present at toxic levels the ratios in potato, before liming, varied from 0·35 to 0·12, and after liming from 1·08 to 0·42.

8. Whilst it has been shown by various workers that the Mn ion may induce Fe deficiency and *vice versa*, there was no clear evidence of these effects being critical in these experiments. Over the range of soils the iron content of the plants did not vary greatly and the determining factor seemed to be the supply of available Mn in the soils.

9. The Fe/Mn ratio did not appear to be of major importance in determining the Mn and Fe status of the field crops studied.

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REVIEWS

THE FRUIT YEAR BOOK, 1948. (The Royal Horticultural Society, London. 1948. Pp. 179+4. Figs. 34. Price 8s. 6d.)

This is the second number of the *Fruit Year Book* to appear under the auspices of the Fruit Group of the Royal Horticultural Society. The signed articles, of which there are some twenty-eight, dealing with many important and some lighter aspects of fruit growing at home and overseas, are contributed by writers whose first-hand acquaintance with the subjects they discuss is a guarantee against the postulation of second-hand ideas. The all too finite space of a brief review ordains that some of the stars of this galaxy, fresh combed from the horticultural firmament, must shine with brilliance unrecorded. This does not infer a lesser magnitude but merely illustrates the eternal truth that two into one won't go.

The book opens with a pleasant sketch of Thomas Andrew Knight by N. B. Bagenal at his most Lytton Stracheyesque. He evidently enjoyed writing it. Reading between the lines of the contribution which follows, it soon becomes apparent that Mesdames Beryl Beakbane and Eleanor Thompson of East Malling (the former, by the way, to be congratulated on the recent award of the Jones-Bateman cup for horticultural research) contrived to have a wonderful, if strenuous, time exploring for plum rootstocks in the more delectable parts of France, and a rather less wonderful one on the same errand in Worcestershire where it rained a lot and the trees dripped. Having kept their eyes open they are able to supplement their travelogue with a highly observant paper on the complicated subject of the intensive training of fruit trees in France. J. M. S. Potter, endeavouring to stimulate the cultivation of pears by the amateur, gives much essential information to that end. Should his enthusiasm lead to an improvement in the English shop pear, that amalgam of wood and grit labelled William if rotten and Comice if just plain hard, he will have pleaded to some purpose. P. Morton Shand writes on English russet apples, a subject on which very little has been published, tracing their history from their first mention by Lyly in 1602 to the present time of their unmerited eclipse. Valuable lists of past and present varieties are given The formation of a Russet Society to preserve what remains from extinction has been mooted. The reduction of our apples to a few standard commercial varieties may mean progress to some but not to Mr. Shand and he says so with entertaining directness. Later he contributes a scholarly article on the large-fruited perpetual strawberries of France with their bearing season of 4 to 8 months, the *fraisiers à gros fruits remontants* of the French catalogues.

In 1944 and '45 East Malling Research Station planted their large walled garden at Bradbourne with apples and pears to show the more important methods of intensive growing and training. Zinita Griffin describes a year's work with these and other fruits there cultivated. E. W. Hobbis entitles his paper " Black currants : Six important points of culture ". These points are hardly of the kind that " have position but no magnitude " for they expand to cover practically everything the black currant grower ought to know. N. H. Grubb writes on " Raspberries for the amateur garden ". The raspberry is so virus-ridden that the planting of certified stocks is a *sine qua non* for success. The few varieties for which such stocks are obtainable are named and commented on. That renowned combination Drs. Kidd and West discuss some problems involved in the storage of mixed varieties of apples and the problems concerning the influence of one variety upon another in matters of ripening or disease and the complex interrelationships involved. Incidentally, this is the *only* paper with a bibliography. From overseas J. P. Hudson writes on "Fruit growing in New Zealand", L. G. Sebire on "Plums and cherries in Victoria", W. R. Leslie on "Canadian prairie horticulture", and H. L. Jaquier on "The fruit garden in Switzerland". Among contributors at home to whom space forbids further reference but whose names alone remove them from the "also-rans" are Raymond Bush (strawberries), Humphrey Denham (apricots), Sir Stephen Tallents (medlar jelly) and A. M. Massee (apple-blossom weevil).

For frontispiece is a vivid portrait of Dr. R. G. Hatton, the first Chairman of the Fruit Group. An appreciation of his work is contributed by M. B. Crane.

G.St.C.F.

THE PLANT IN HEALTH AND DISEASE. By W. A. R. Dillon Weston and P. Eric Taylor. Foreword by J. A. Scott Watson. (London: Crosby Lockwood & Son, Ltd. 1948. Pp. 173. Price 21s.)

This addition to Messrs. Crosby Lockwood's Agricultural and Horticultural Series is not another textbook of plant diseases but develops, in essay form, a comprehensive and richly illustrated account of the application of the science of plant pathology in its widest sense to the problems of increased crop production.

The authors, who are well-known practitioners in plant pathology, wisely draw their illustrative material mainly from their own sphere of long experience in what is now the Eastern Province of the recently established National Agricultural Advisory Service. The diseases selected for detailed treatment are therefore mainly those of the agricultural (cereal and root) and market garden crops.

An introductory sketch of the earliest recorded human reactions to and against crop "plagues" (Chapter I) leads to the comparatively recent discovery of the role of micro-pathogens (II) and the stage is then set for plant pathology in its modern sense. But before considering the plant pathogens in greater detail, an account is given of the developing green crop plant, from the seed (with its hereditary potentialities) onwards, in its normal soil and aerial environment of dead matter and other living organisms, and of the range of pathological effects directly attributable to deviations from that normality (III, IV).

The authors then return to a more detailed treatment of the pathogenic fungi, their structure, modes of spore dispersal and classification (V, VI) and, in the light of this, they survey the wide range of pathogenic host interactions and the varied problems of diagnosis and control. Here specimen case-histories are provided (VII).

The reader is now ready to consider in greater detail the individual diseases and, starting logically with seed-borne diseases, a general discussion of the evolution of the methods, specifics, and machinery of seed treatment (VIII) is followed by an account of the application of such methods to specific seed-borne disease of cereals and of other crops (IX and X). But the seed may not only carry the "germs" of its own disease; it and the seedling are also liable to parasitic and non-parasitic depredations from the outside soil environment. The causes of "soil sickness" are therefore reviewed in Chapter XI and examples of diseases caused by soil-inhabiting fungi follow (XII).

At this stage the complexity of the crop-pathogen-environment relations is becoming apparent and in Chapter XIII the various facets of these relations are exhibited by reviewing all the diseases of a single glasshouse crop, the tomato, the cultural environment of which is subject to a high degree of artificial control. The review leads on from diseases originating in the soil to aerially distributed diseases (fungus, bacterial and virus) and to the non-parasitic or physiological diseases and these latter categories are dealt with in greater detail in the following three chapters (XIV-XVI).

Two concluding chapters (XVII-XVIII) describe existing legislation against crop diseases and the organization of the services for technical advice available to growers in this country. Two Appendices provide respectively a tabular classification of specimen diseases of each crop plant according to the nature of their causes, and a useful list of reference books on specific classes of diseases and crop plants.

Finally, special mention must be made of the series of six text figures and of sixty photographic plates which are admirably executed, selected and reproduced and which form an integral and essential part of the work. Some of the illustrations of the pathogenic fungi are photographic reproductions of glass models executed by the senior author.

The volume should fulfil a need in providing a most fascinating and readable introduction to plant pathology for students and growers, and a stimulating " refresher " for the practitioner himself. An index is provided.

R.V.H.

STUDIES OF THE PHYSIOLOGICALLY-ACTIVE VOLATILE ORGANIC COMPOUNDS PRODUCED BY FRUITS :

II. THE RATE OF PRODUCTION OF CARBON DIOXIDE AND OF VOLATILE ORGANIC COMPOUNDS BY KING EDWARD VII APPLES IN GAS STORAGE, AND THE EFFECT OF REMOVAL OF THE VOLATILES FROM THE ATMOSPHERE OF THE STORE ON THE INCIDENCE OF SUPERFICIAL SCALD

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SYNOPSIS

The present method of controlling superficial scald of apples in gas storage by wrapping them in paper containing oil is usually very effective but is costly. This is mainly because it requires so much labour. It also takes time and delays the closing of the store. Can simpler, equally effective methods be found ? It was expected that removal of part of the volatile vapours given off by the apples, by circulating the storage atmosphere through a gas-mask type of filter, would reduce scald. This expectation was based on the theory that scald is caused by the accumulation of one or more of these volatiles in the storage atmosphere. Suitable filters were developed and experiments in which the storage atmosphere was analysed showed that these filters did remove a very large part of the apple volatiles. Nevertheless, it was found that the amount of scald occurring on unwrapped King Edward VII apples in gas storage was almost the same in a store with filters as in one without.

The well-known effect of gas storage in lowering the rate at which apples lose CO_2 is shown in the present paper by means of diagrams. It is this condition which is responsible for the extension of storage life of apples in gas mixtures.

INTRODUCTION

THE first paper in this series (Fidler, 1948) described the results of a survey of the concentration of volatile organic compounds in commercial gas stores containing apples. It was shown that the volatiles can be separated into two fractions, namely ethylene and " odorous " (or non-ethylenic) volatiles. The concentration of ethylene often reached high values but the odorous volatiles were present in lower concentration. The technique did not allow the rates of production of the volatiles to be estimated accurately. The present paper describes experiments with King Edward VII apples on a semi-commercial scale in the laboratory during the seasons 1946-7 and 1947-8, and gives figures for the rates of production of volatiles, of carbon dioxide, and of uptake of oxygen.

The historical introduction to the previous paper discussed briefly the relationship between the volatiles and the disease known as " superficial scald " of apples and indicated that the effect of removal of the volatiles from the atmosphere of the store was being investigated. This paper constitutes a progress report on the results of experiments (which are being continued) in which apples were stored under normal gas storage conditions, both wrapped in oiled paper and unwrapped, and in gas mixtures from which either all the volatiles, or the odorous fractions only, had been removed. The results are not completely in accord with the generally accepted theory for the cause of superficial scald.

It may be said at once that from the practical standpoint, there is no immediate substitute for the oiled wrapper as a means of controlling superficial scald of apples in gas storage.

The first section of this paper describes the experimental methods and presents the results, with some general discussion of their less important implications. The second section ("Discussion") deals with the more important conclusions and their bearing on the problem of scald.

EXPERIMENTAL.

A. APPARATUS AND METHODS.

The objective was to reproduce, as nearly as possible, in the laboratory, the conditions in a good commercial gas store, i.e. the fruit was to be loaded into the stores directly after picking, and cooling and accumulation of the CO_2 of respiration were to proceed simultaneously. The correct concentration of CO_2 was maintained by ventilation with air only, so that the concentrations of volatiles were the same as in commercial practice.

Four special gas-tight cabinets were built of welded mild steel sheet, on frameworks of angle iron. Each cabinet held sixty standard sized (10½ in. × 12 in. × 10½ in. external) bushel boxes, in six layers of ten boxes each.

Each cabinet had, internally, a false floor and ceiling made from perforated steel sheets, and false end walls of undrilled sheet, leaving an air duct 2 in. × 40 in. in cross section down each end and along the floor. At the apex of a pyramidal plenum above the false ceiling was mounted an axial flow fan, delivering into a duct which led into the two side air ducts. Thus there was a flow of air from the fan, down the side ducts and up through the floor and the boxes.

The cabinets were loaded through a door which could be sealed by means of a steel gasket faced with rubber on both surfaces. The door was bolted down by screws passing through the gaskets and the door frame.

The boxes had slatted bottoms, to permit easy flow of air, and they fitted so closely into the cabinets that most of the air flow must have been over the fruit.

The fan delivered 650 cubic feet of air per minute, free-running, but the resistance of the side ducts and the ceiling and floor perforations reduced the flow to about 380 c.f.m., which was equivalent to a rate of flow, in the empty cabinet, of 22 feet per minute, and to four changes of air per minute in the central space. Mr. G. Mann* has calculated that the resistance of the boxes further reduces the airflow to 150 c.f.m., or a linear speed of 9 feet per minute. This is much higher than is usual for a commercial store; typical gas stores have fans which give about 20 changes per hour, measured on the empty store.

In the centre of the front and back walls of the cabinets were ¼-in. pipes for admitting air and withdrawing gas, and alternative connections could be made to valves fitted on the roof, communicating with either the suction or delivery sides of the fan.

Two of the cabinets were fitted with centrifugal fans blowing air through filters. The object of the filters was to remove as much as possible of the odorous volatiles from the atmosphere of cabinet No. 1, but to leave the ethylene behind, and to remove both ethylene and the odorous fractions from the atmosphere of cabinet No. 2. Walls (1942) has shown that active carbon will remove the odorous fraction, but does not adsorb ethylene at ordinary temperatures. Active carbon was therefore used in the filter on cabinet No. 1. The ethylene and odorous volatiles could have been removed from the cabinet No. 2 by burning them in a furnace with copper oxide, but it was not desired to complicate the problem of refrigeration by doing this. Smock (1943, 1944, 1945), Southwick (1945, b), and Smock and Southwick (1945) report that ethylene is removed by charcoal impregnated with bromine. Smock (1944) states that charcoal containing 25 per cent. of bromine does not lose bromine on aeration. In the present experiments it was found that charcoal containing 20 per cent. by weight of bromine smelt very strongly of bromine, and further, that the impregnated charcoal was very corrosive. It quickly corroded many metals, including monel and stainless steel; tin was the only metal tried which was at all resistant to attack. Accordingly the filter container was at first made from non-metallic material. After tests a suitable material was found in polymerized ethylene sheet (sold under several trade names). This is inert, resistant to attack by bromine, and easy to make into any desired form. Retaining gauzes were made from glass-cloth. It was later found possible to use copper coated with a thermosetting bakelite resin for the filters, with gauzes of tinned steel wire. The plain carbon was housed in steel or copper filters.

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* From the data in his possession on the resistance to air flow of a stack of potatoes.

The filter systems and their associated motors have been sources of trouble in both seasons. In 1946-47 the fans with their driving motors were housed in gas-tight boxes outside the cabinets, with the filters mounted directly on top, drawing gas from the suction side of the circulating duct and delivering it to the delivery duct. The plain carbon filters were standard service gas-mask fittings, containing "briquette" carbon (which is made from coal). The brominated carbon filters contained 135 gm. of brominated carbon (depth 1½ cm.), followed by 135 gm. of plain carbon (either "briquette" or "coconut shell" was used at different times) to adsorb bromine vapour. It was not possible to obtain induction motors in time, so brush-type motors had to be used; these gave a good deal of trouble. Further, vibration tended to loosen the joints on the filter circuit, and leakages occurred at times. The brominated carbon rapidly lost its efficiency as an ethylene absorbent, and needed frequent renewal.

New centrifugal fans were obtained for the 1947-8 season, driven by induction motors. The motors were reputedly of low wattage, and it was decided to house motors and filters inside the cabinets, where they occupied the space of two boxes at one lower corner of the stack. The fan drew gas through a hole in one side duct, and discharged it through the filter directly upwards to the boxes above. This arrangement cut out leakage, but as the motors consumed about three times their rated wattage, led to difficulties in cooling the cabinets. The plain carbon filter was filled with a mixture of briquette and coconut shell carbon (weight 1,400 gm., depth 10 cm.). The brominated carbon filter held 1 kg. of brominated carbon, containing 23 per cent. by weight of bromine, followed by 500 gm. of plain carbon. These filters needed renewal much less frequently than the small ones used in 1946-7.

The rate of flow of gas through the filters was 3·3 c.f.m. in 1946-7, and 44 c.f.m. in 1947-8, equivalent to 1·7 and 23 changes of air per hour respectively, calculated on the space of the empty cabinet.

The cabinets stood in a large refrigerated chamber, cooled by wall grids, and fitted with small fans for air circulation. Each cabinet was fitted with six thermocouples, one in the plenum, and five spaced out among the fruit. The temperatures were measured and recorded daily.

At the beginning of the experiments, the cabinets were loaded with weighed boxes of apples (the total weight of fruit ranging from 1,955 to 2,155 lb. per cabinet), the doors were sealed, and the cabinets tested for gas-tightness by inflation with air to a pressure of 2 in. of water and painting of all joints with a soap solution. Cooling and slow aeration were then begun.

All four cabinets were ventilated in the same way. A pipe led fresh air from outside the building into the tube at the back of each cabinet, at a rate determined by the rate of withdrawal of the atmosphere through the sampling tube on the opposite face of the cabinet. The emergent gas-stream was divided, one stream passing at 4 to 5 litres/hour through a Venturi flow-meter, then through a Haldane gas-sampling tube, and finally through the volatile absorption train. This consisted of two Dreschel bottles, with sintered glass spoons (porosity No. 1), containing plain or "activated" sulphuric acid (i.e. sulphuric acid plus silver sulphate, see Fidler, 1948). Odorous and ethylenic volatiles were estimated in these acids by the methods already described in the first paper of this series. The rate of flow was controlled by a mercury manostat. At the beginning of the experiment, while the carbon dioxide was accumulating inside the cabinet, this suction line was the only one operating, but as soon as the concentration of CO_2 reached 8 to 9 per cent., the second stream was started. This led from the cabinet, through a Venturi meter reading from 5 to 30 litres/hour, to an adjustable manostat.

The gas-samples were at first analysed daily and later at two to four day intervals, in a Haldane gas-analysis apparatus. If the concentration of CO_2 was lower than desired, or tending to fall, the rate of ventilation by the second (or faster rate) suction line was reduced by varying the setting of the manostat. Conversely, the suction rate was increased if the concentration of CO_2 tended to rise.

The acids from the volatile-absorption traps were analysed weekly for the first few months, and at ten-day intervals thereafter.

Thus the primary data consisted of: (a) the concentration of CO_2, and occasionally of O_2, in the atmosphere of the cabinets, (b) the total volume of gas withdrawn from the cabinets (and thus of air admitted), (c) the volume of gas passing through the acids, and (d) the weights of carbon representing ethylene and odorous fractions of the volatiles. In addition, the weight of apples, and the volume of free space not occupied by apples or boxes inside the cabinets were known.

These data were sufficient to allow calculation of (a) the rates of production of CO_2, of ethylene, and of the "odorous" volatiles, (b) the concentration of volatiles in the storage atmosphere, and (c) the respiratory quotient.

In addition to the experiments in these large cabinets, the work in the second season included (a) measurement of the rate of production of CO_2 by a small 3 kg. sample of apples by the usual method, (b) the measurement of the rates of production of CO_2 and of volatiles by a 50 kg. sample in a small metal cabinet, in low concentration of CO_2,* and (c) a 50 kg. sample kept in 8-9 per cent. CO_2 under conditions similar to those of the control sample in the large cabinet, but without any mechanical circulation of air over the fruit.

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* The objective was to determine the rates of production of the volatiles in air. In order to obtain reliable figures a fairly large quantity of fruit had to be used. The limit to the rate of aeration was set by frothing of the acid in the Dreschel bottles, and in practice the mean concentration of CO_2 in the "air" was about 1 per cent.

B. APPLES USED.

It was necessary to use a variety of apple liable to develop scald in gas storage, and preferably one which is normally gas stored in large quantities. The Bramley's Seedling apple does not normally fit the first requirement, but because of its commercial importance it was used in 1945-6 for preliminary trials. Bramley's which were notorious for developing scald were obtained, but they rotted so badly in prolonged storage that it was decided to use a different variety in the following year.

Lane's Prince Albert apples would have been used, but the Kentish crop of this variety was so light in 1946-7 that no farmer could spare 4 tons from one orchard. King Edward VII apples grown at Marden, Kent, samples of which had developed scald in 1945-6, were used. They were picked somewhat earlier than the normal date in order to increase susceptibility to scald.

In 1946, the apples were picked on 30/9/46, sorted and packed into the weighed boxes on 1/10/46, and loaded into the cabinets from 9.30 a.m. until 2 p.m. on 2/10/46. The cabinets were sealed by 3.15 p.m.

In 1947, picking and sorting were done on 22 and 23/9/47, and the cabinets were loaded and sealed by 7 p.m. on 23/9/47. The apples were larger than those obtained from the same trees in 1946.

C. RESULTS.

In presenting the results, the cabinets will be referred to as Cabinets 1, 2, 3, or 4. All were run with a mean concentration of CO_2 of about 8 to 9 per cent., and the treatments were as follows:

Cabinet 1: Unwrapped apples, plain active carbon filter.
,, 2: ,, ,, brominated active carbon filter.
,, 3: Apples in oiled wrappers. No filter.
,, 4: Unwrapped apples. No filter.

It is usually desirable to compare a given graph for one cabinet with those for the other three. Thus all four should be plotted on the same axes. In order to reduce confusion, the results are presented in the form of smoothed curves. Since there is no reason accurately to correlate any two of the quantities presented, this method of treatment is not misleading. An appendix to this paper gives two graphs of the actual values obtained in typical experiments, so that the legitimacy of the smoothing may be checked.

1. *Efficiency of the system of cooling*: The cabinets and their associated apparatus are bulky, and had to be accommodated in a large cool store. The only large store available was cooled by brine grids on the walls, and the spatial distribution of temperature was not perfectly uniform. In each season this has been reflected in the mean temperature of the apples in the four cabinets, but the differences were not at any time very serious. Thus, in 1946, all four lots of fruit cooled at a uniform rate. The temperature was 64° F. on loading, falling to 46° F. on the second day after loading, and 40° F. by the fourth day. The mean temperatures of the apples for the whole 204 days of the experiment were: Cabinet 1, 39·4° F.; Cabinet 2, 39·1° F.; Cabinet 3, 38·5° F.; and Cabinet 4, 38·6° F. The difference between

the warmest and coolest parts of any cabinet never exceeded 1° F. The mean temperature of the room was about 37° F., with a maximum spatial difference of ±1° F.

As has already been mentioned, the centrifugal fans for the filters were housed inside Cabinets 1 and 2 in the 1947-8 season, because of trouble with leaks in the previous year. Whereas the input of energy had been expected to be about 70 watts/hour, it was actually three times this figure. This caused a rise in temperature of the apples in Cabinets 1 and 2, relative to those in Cabinets 3 and 4.

The initial temperature of the apples was 57° F. The temperature record is summarized below in Table I.

TABLE I.

Mean temperature of apples in Cabinets 1 to 4, 1946-7.

(*See notes below Table I.*)

Time	Cabinet: Temperature ° F.							
	1			2			3	4
	Mean.	Mean of warmest boxes.*	Mean of other boxes.†	Mean.	Mean of warmest boxes.*	Mean of other boxes.†	Mean.	Mean.
Day 2 ..	49·0	—	—	48·2	—	—	46·3	44·1
4 ..	46·0	—	—	45·2	—	—	41·0	41·0
6 ..	42·6	—	—	42·0	—	—	38·7	39·0
8 ..	44·8	—	—	44·1	—	—	40·2	40·2
10 ..	40·8	—	—	40·1	—	—	38·0	37·7
14 ..	40·7	—	—	39·9	—	—	37·8	37·4
20 ..	41·6	45·9	40·7	41·3	46·9	40·2	39·3	39·6
Days 20 to 239	40·8	45·3	39·9	40·9	46·7	39·7	37·9	38·1

Mean temperature of chamber: 36·3° F.
Maximum deviation between warmest and coolest parts of No. 3 and 4: 1° F.

* Thermocouple records show that these occurred in the column of boxes immediately above the filter, and at the top of the two adjacent columns.

† The rest of the boxes in Cabinets 1 and 2 differed from each other by not more than 1° F.

Thus the mean temperature of Cabinets 3 and 4 was about 38° F., but Cabinets 1 and 2 each contained apples with mean temperatures of about 40° F. and 46° F. These could be identified at the end of the experiment but it must be remembered that the warmer apples affected the levels of the mean curves for production of CO_2 and volatiles.

Graph D in Fig. 3 gives the smoothed curve for the mean temperature of Cabinet 2 in 1947-8.

2. *Production of carbon dioxide:* In the 1946-7 season, Cabinets 3 and 4 were sealed without any difficulty, but the seals on the door and filter of Cabinet 1 and the filter of Cabinet 2 were at first imperfect. Thus these cabinets did not accumulate CO_2 or attain a steady state quite as quickly as Cabinets 3 and 4 (as shown in Table 2), and the early values for rate of production of CO_2 (see Fig. 1) are too low.

TABLE II.

Accumulation of CO_2 in cabinets, 1946-7.

Day.	Cabinet : CO_2 %.			
	1	2	3	4
2	4·5	4·7	5·1	5·5
4	6·2	7·0	7·8	8·4
6	7·3	8·4	9·4	10·2
9	8·9	8·3	8·9	9·4
12	10·1	8·9	8·2	8·6
15	9·0	8·8	8·1	8·5
15 to 204	8·5 to 9%, all cabinets.			

In 1947-8 no trouble was encountered from leakage and all four cabinets accumulated CO_2 at the same rate (except that Cabinet 1 had 1 per cent. more than the others on the fifth day). Graph C in Fig. 3 shows the values for the first 15 days. The mean concentration from day 15 to day 239 was 8½ for all cabinets, except that it fell to about 6 per cent. in Cabinet 2 when the filter was changed (reaching 8 per cent. two days later).

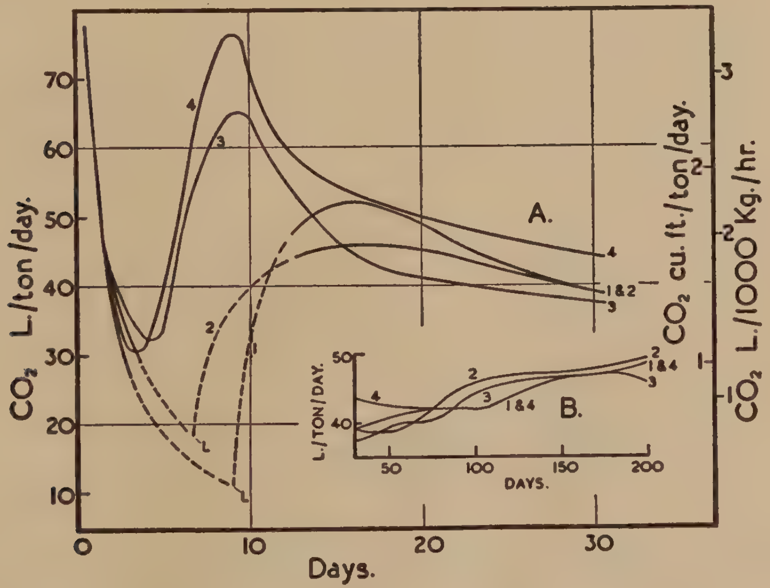

FIG. 1.

Rates of Production of CO_2 by Apples in Gas Storage, 1946-47.

Curves A. Days 0-30. L indicates leaks detected.
,, B (inset). Days 30-204. Note altered time scale.
Cabinet No. 1. Plain C filter.
,, No. 2. Brominated C filters.
,, No. 3. Oiled wrappers.
,, No. 4. No wrappers.

The mean concentration of oxygen in the cabinets was about 13 per cent., in both seasons.

When it was decided to attempt to measure the rate of production of CO_2, nothing better was hoped for from the technique adopted than rough estimates of the course of the graphs. Actually, the graphs proved to be as smooth as those obtained in the laboratory by more conventional methods with small samples of fruit. The results for 1946-7 are given in Fig. 1 and for 1947-8 in Fig. 2.

In these Figures the values for the first 30 to 40 days have been plotted on an open scale, with values for succeeding days on a compressed time scale. This permits the whole curve for each cabinet to be presented on one chart, and the early portion to be studied in detail.

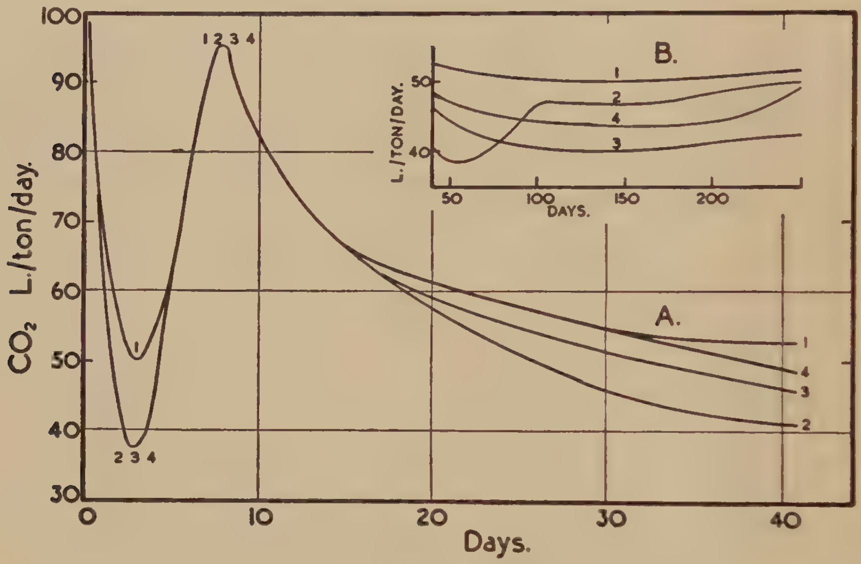

FIG. 2.
Rates of Production of CO_2 by Apples in Gas Storage, 1947-48.

Curves A. Days 0-40.
,, B (inset). Days 40-239. Note altered time scale.
Cabinet No. 1. Plain C filter.
,, No. 2. Brominated C filter.
,, No. 3. Oiled wrappers.
,, No. 4. No wrappers.

The rate of respiration is expressed as litres of CO_2 per ton of apples per day. This hybrid expression has the merit of giving a reasonably large whole number, and is in fact very close to litres/1,000 kg./day. From the practical angle cu. ft./ton/day would be a useful unit. This is given on the right abscissa, together with the metric units, litres/1,000 kg./hour.

In Fig. 1, the graphs for Cabinets 1 and 2 are unreliable for the first 12 days; as shown, they include a period when the cabinet was leaking, and these leaks, particularly when between the blower and the filter, led to loss of CO_2. From about day 12 onward they are as reliable as those for the other cabinets.

But to what extent do the graphs represent either the actual levels or the relative levels of production of CO_2 from one day to another? Are the inflexions in the curves indicative merely of physical changes in the system, or do they represent true biological changes?

The physical changes were that the apples were transferred from air to atmospheres in which the concentration of CO_2 was rising daily for the first six days, while at the same time the temperature was falling steadily from about 60° F. to 40° F. Both these factors should have increased the amount of carbon dioxide held in solution in the cell sap. The pH of the cell sap is so low that solution can be the only process responsible for all the CO_2 produced in respiration not being given off by the fruit. It is possible to make an estimate of the magnitude of the effect of increased solubility on the apparent rate of production of CO_2. The first assumption is that the apple behaves as if it were a mass of water weighing about 85 per cent. of the weight of the fruit. Walker, Lewis, McAdams, and Gilliland (1937) give a graph derived from Bohr and Bock (1891)* for the solubility of CO_2 in water at different temperatures. The solubility is directly proportional to the partial pressure. Unpublished data obtained by W. H. Smith, of the Ditton Laboratory, show that the internal concentration of CO_2 in the intercellular spaces of an apple is increased by about 1 per cent. for each 1 per cent. increase in the external concentration. Thus one may calculate, for any period of time, how much given changes in external concentration of CO_2 and of temperature should affect the amount of CO_2 in solution, and thus the amount apparently produced by the apple in that period. Actually there exists a record of the solubility of CO_2 in the Cox's Orange Pippin apple at one temperature (Hardy, 1939). This gives a value of 1·63 to 1·69 ml. CO_2/mm. partial pressure/kg. of apples, at 39° F. The value calculated as outlined above is 1·63.

Graphs A and B of Fig. 3 show respectively the observed and corrected values for the production of CO_2 in an experiment (Cabinet 2, 1947-8), and Curves C and D give the data for the accumulation of CO_2 and fall in temperature over the same period. It will be seen that the two respiration curves are similar in shape, but that the actual rate of production of CO_2 was initially rather higher than the observed rate of escape. By the tenth day, when the concentration of CO_2 in the atmosphere and the temperature of the fruit were stable, all the CO_2 produced in respiration escaped from the fruit.

Thus while the observed initial rates of production of CO_2 may be lower than the actual rates, any deductions drawn from the shape of the graphs will relate to true biological changes in the system. Differences between the levels of the four graphs in either Fig. 1 or Fig. 2 may be due to errors of sampling and estimation, or to true differences between the rates of respiration. The graphs are fully discussed in the next section, but the following gives some indication of their significance. The graphs of Fig. 1 are, after the 25th day, never separated by more than about 5 to 6 CO_2-units; they are regarded as being at approximately the same levels. Fig. 2 shows wider divergences after the 30th day. The graphs for Cabinets 3 and 4 are regarded as being identical. The higher level for Cabinet 1 is considered as due to the effect of temperature differences (previously noted), and the respiration of the apples in Cabinet 2 is considered to be significantly lower than that of those in Cabinet 1 from about the 25th to the 100th day.

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* Wied. Ann., **44**, 318.

There is one further consideration, does any of the CO_2 originate from the active carbon, in Cabinets 1 and 2? Walker *et al.* (loc. cit.) quote Lowry and Hulett's (1920)* figure of 35 litres of CO_2 adsorbed per kg. of carbon at an unspecified pressure or temperature. This at least indicates that carbon can adsorb CO_2, and may release it again. Tests in the laboratory have shown that plain carbon gives off a negligible amount of CO_2 when aerated (about 6 mg./kg. carbon/day, at 65° F.). Freshly brominated carbon gives off about 100 mg./kg./day for the first day, falling to about 6 mg./kg./day thereafter. Possibly bromine displaces some adsorbed CO_2. The initial liberation of CO_2 would have affected the curves for CO_2-output by 0·15 per cent. in 1947-8, and about 0·02 per cent. in 1946-7, in other words, not at all.

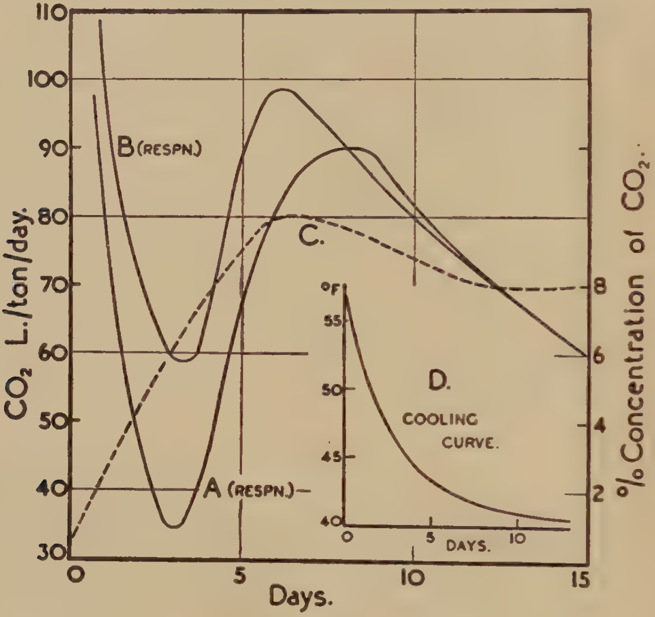

FIG. 3.

Cabinet No. 2. 1947-48.

Curve A. Observed rate of production of CO_2.
,, B. Rate of production of CO_2, corrected for solubility of CO_2 in sap.
,, C. Accumulation of CO_2 in the cabinet.
,, D. Mean temperature of apples in cabinet.

The rate of production of CO_2 in air in 1947-8 is given in Fig. 4A, graph (a). At the beginning of storage the respiration of the apples had not yet entered on the climacteric phase. Respiration records of the apples used in 1946-7, at 60° F. (but not given here), showed that these apples too were pre-climacteric.

3. *Respiratory Quotient:* During the 1946-7 season, estimates of oxygen uptake were made, and the R.Q. was calculated. Table 3 summarizes the results. The apples in all four cabinets behaved similarly.

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* J. Amer. Chem. Soc., 42, 1393.

TABLE III.

Mean values of the R.Q. of King Edward VII apples stored in 8 per cent. CO_2 at 38° F.

Days in gas ..	5	10	20	30	50	100	200
R.Q.	0·80	0·90	1·00	1·07	1·12	1·10	1·03

The value for day 5 will be low because of solution of CO_2 in the cell sap, and that for day 10 may possibly be somewhat on the low side for the same reason. But that for day 20 suggests suppression of respiratory loss of acid. Acid loss appears to have begun again by day 30, and thereafter the R.Q. values are normal for apples in air. It would be interesting to have data for the loss of carbohydrates and acid during the early part of gas storage.

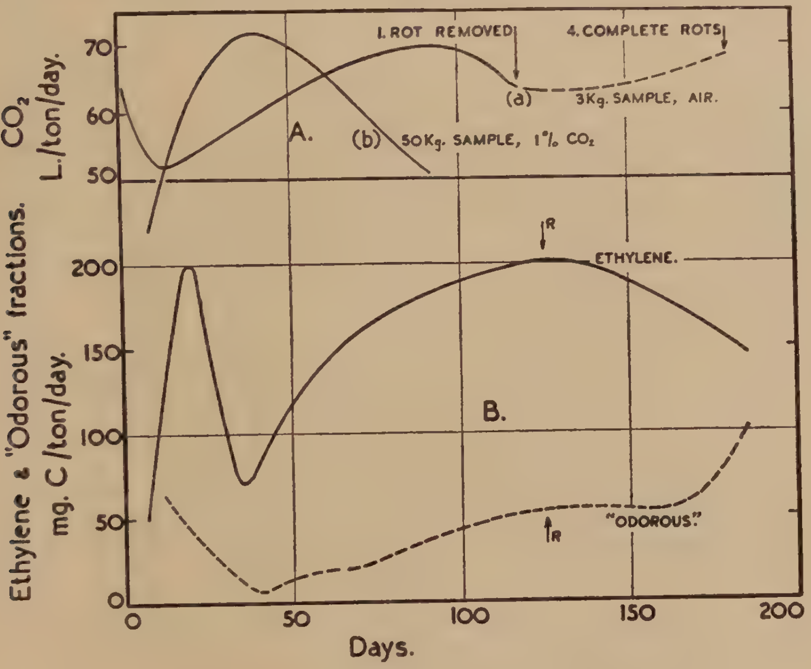

FIG. 4.

Apples in Air or Very Low Concentration of CO_2 (about 1 per cent.). 1947-48.

Curves A. Rates of production of CO_2.
,, B. Rates of production of volatiles by the 50 kg. sample in 1 per cent CO_2. (R ↑ indicates rotting becoming serious.)

No measurements of the R.Q. were made in the 1947-8 season.

4. *Production of volatiles:* The concentration of the volatiles in the atmosphere of the cabinets is given in Figs. 5 and 6, and the rates of production are shown in Figs. 7A and 7B. Different scales were used in plotting the two fractions in order

more clearly to show the shape of the graphs for odorous volatiles. In Figs. 5 and 6 the units used are (as in the first paper) " mg. C/100 L. gas " ; using the conventions described in the first paper, concentrations have also been calculated as " parts per million ", and the right abscissa carries a scale of these units.

Because of the presence of the filters, one cannot calculate the rates of production of ethylene for Cabinet 2, or of odorous volatiles in Cabinets 1 and 2.

The most striking result (shown most clearly on Figs. 7A and 7B) is that the production of ethylene, which increases rapidly in the first weeks of storage, continues at a high level throughout the whole period of storage. This result is contrary to expectation. Fuller discussion of the significance of the shapes of the graphs is deferred to the next section.

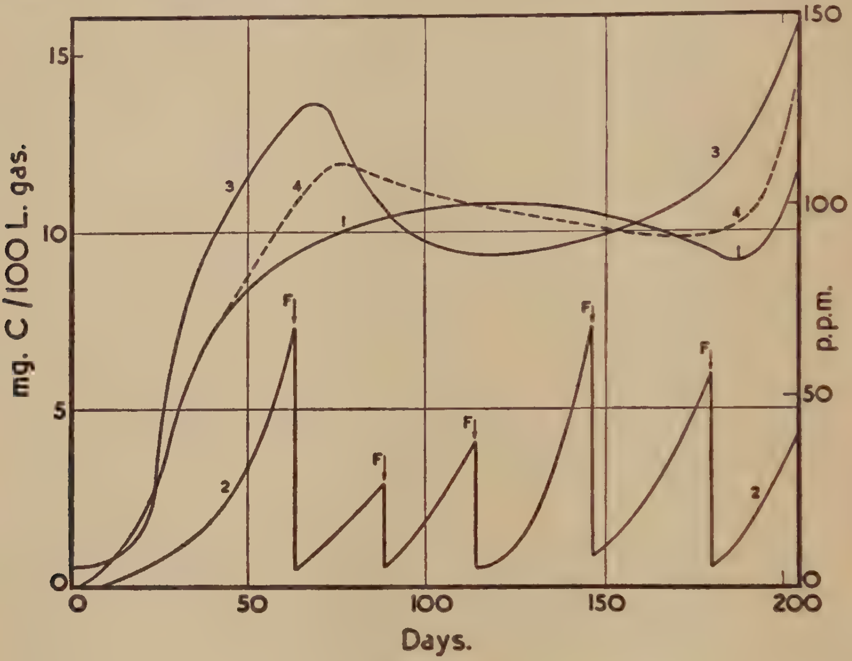

FIG. 5A.

Concentration of Volatiles (Ethylene) in the Cabinets. 1946-47.

Cabinet No. 1. Plain C filter.
,, No. 2. Brominated C filter.
,, No. 3. Oiled wrappers.
,, No. 4. No wrappers.
F↓ indicates renewal of brominated C filter.

Differences between the levels of the graphs are mainly due to the filters, the efficiency of which will be considered here. This can be gauged by comparing the graphs for ethylene in Figs. 5A and 6A for Cabinets 1 and 2 with those for Cabinets 3 and 4, and similarly for non-ethylenic volatiles in Figs. 5B and 6B.

Considering the plain carbon filter (Cabinet 1) first, it will be seen that the graph for ethylene in Cabinet 1 (Fig. 5A) is significantly the same as that for Cabinet 4, which contained unwrapped apples and had no filter. This means that the briquette carbon did not adsorb any significant amount of ethylene. Similarly (Fig. 6A) the graphs for ethylene are substantially the same for Cabinets 1 and 4 in the following season. Thus neither briquette nor coconut shell carbon adsorbed ethylene. Smock (1944) quotes Munkelt (1937) as suggesting that coconut shell carbon may adsorb ethylene. What Munkelt states is that it is a more efficient adsorbent for many vapours than ordinary active carbon. There is no record of an untreated carbon which will, at ordinary temperatures and pressures, adsorb significant amounts of ethylene. Patented systems in which plain activated carbon is used as an adsorbent for this gas operate at very high pressures, or very low temperatures.

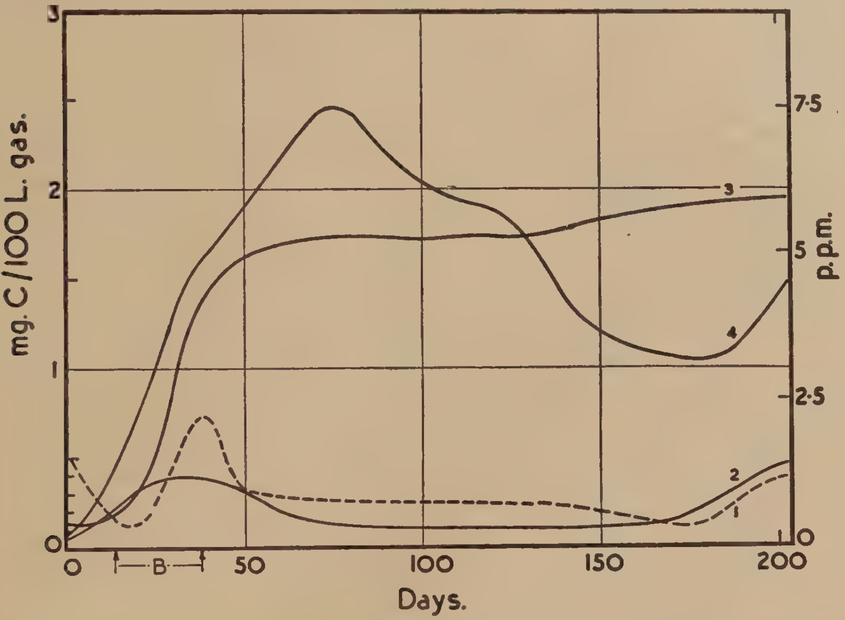

FIG. 5B.
Concentration of Volatiles (" Odorous ") in the Cabinets.
1946-47.
Zone B. Frequent breakdown of motors fitted to filters.

The plain carbon was, however, an efficient adsorbent of the non-ethylenic fractions (see Figs. 5B and 6B). In 1946-7, from the 25th to 50th days (when the centrifugal fan frequently broke down), the level of these volatiles in Cabinet 1 was about 0·25 mg. C/100 L. This was about one eighth to one sixth of that present in the two cabinets (3 and 4) without filters. The filter did not appear to be quite so efficient in 1947-8, when the level of " odorous " volatiles rose steadily from 0·1 to 0·6 mg. C/100 L. over 239 days. But for the first 150 days it reduced the level of these volatiles by about 80 per cent. The filter used in the second season contained a larger mass of charcoal, through which the gas circulated more frequently than in the first season. It was expected to be more efficient. At this stage of the argument, the presence in the " odorous " fraction of a constituent which passes through active charcoal, yet is absorbed in the plain sulphuric acid trap, cannot be ruled out.

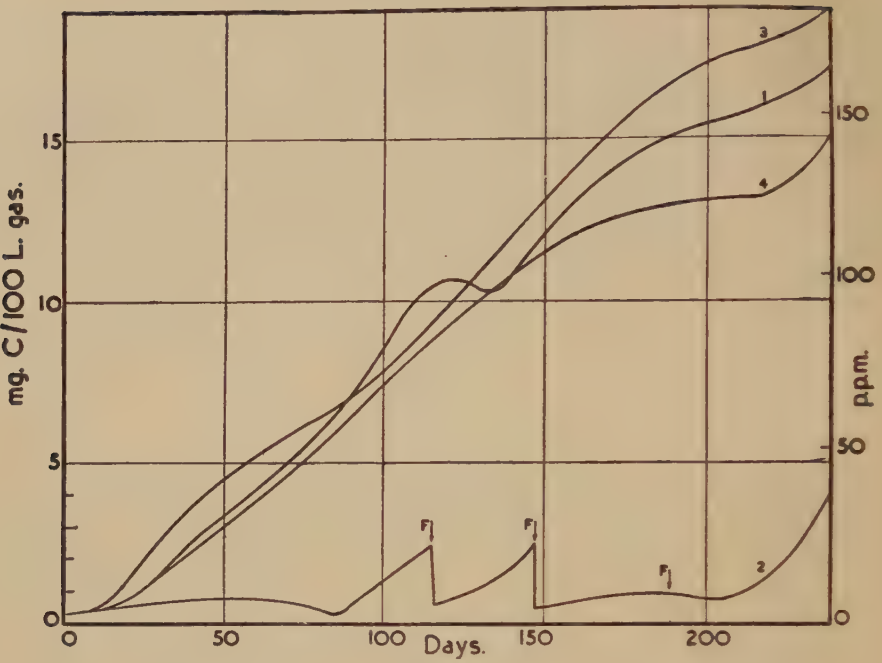

FIG. 6A.

Concentration of Volatiles (Ethylene) in the Cabinets.
1947-48.

F↓ indicates renewal of brominated C filter.

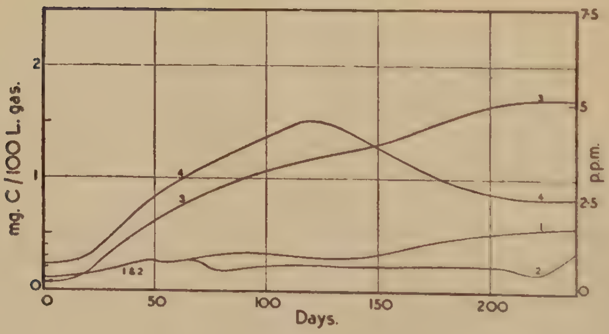

FIG. 6B.

Concentration of Volatiles ("Odorous") in the Cabinets.
1947-48.

Cabinet No. 1.	Plain C filter.	Cabinet No. 3. Oiled wrappers.
,, No. 2.	Brominated C filters.	,, No. 4. No wrappers.

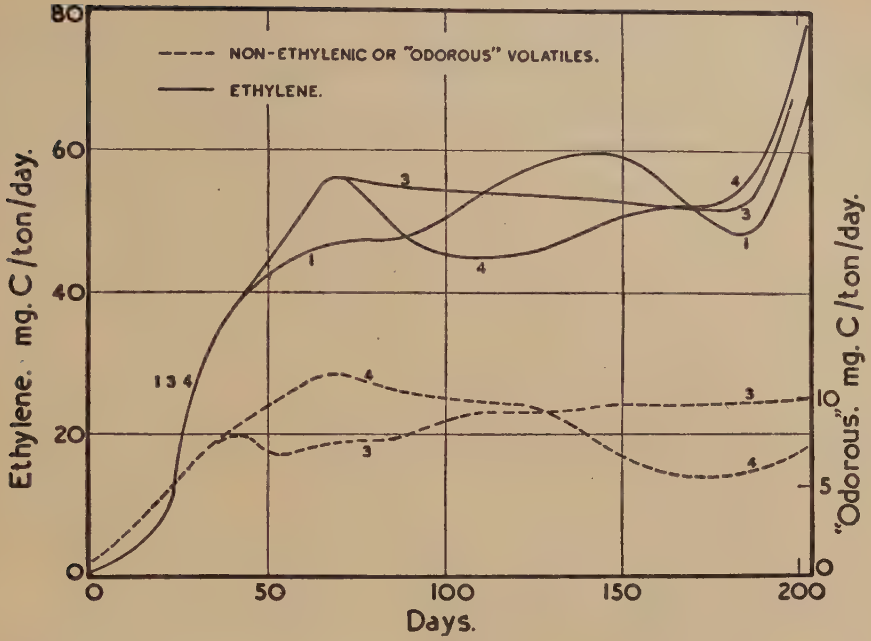

FIG. 7A.

Rates of Production of Volatiles by Apples in Gas Storage. 1946-47.

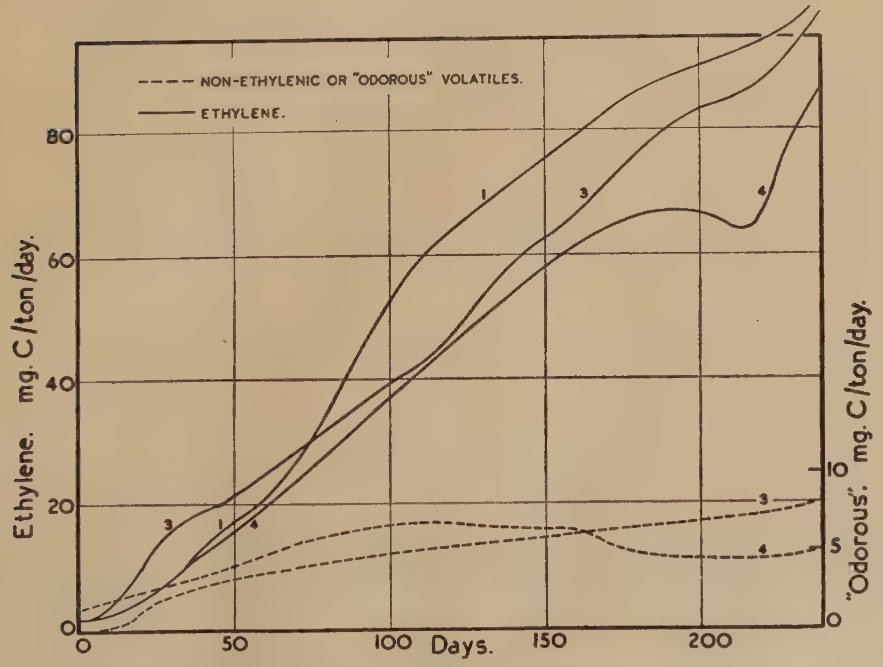

FIG. 7B.

Rates of Production of Volatiles by Apples in Gas Storage. 1947-48.

Consider now the brominated charcoal filter. This was introduced to remove ethylene as well as the non-ethylenic volatiles. It has been found to be far from ideal for the purpose, although reasonably good removal was obtained with it in the 1947-8 season. Smock and Southwick (loc. cit.) state that 1½ lb. (650 gm.) of carbon containing about 23 per cent. bromine are sufficient to absorb* the ethylene from about 5 tons of apples per season. This is for air storage, and a comparison of Fig. 4B and Fig. 7 shows that apples produce ethylene at a faster rate in air than in gas-storage. Thus the 135 gm. used in 1946-7 for a ton of apples in gas-storage should have been adequate, on the basis of Smock's recommendation. The kilogram of brominated carbon used in 1947-8 should have been sufficient for about seven times the weight of apples used. Based on the formation of ethylene dibromide, and taking

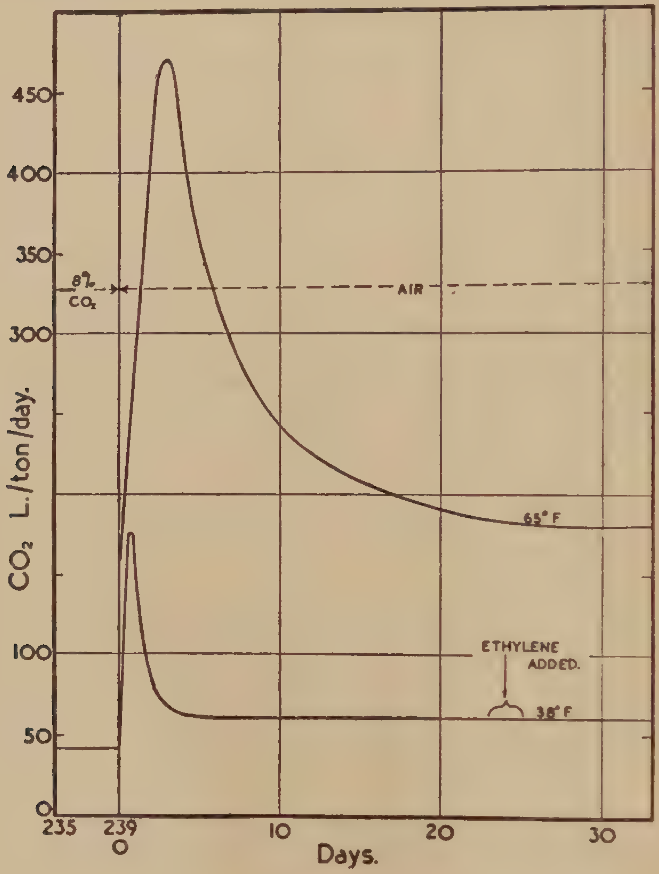

FIG. 8.

Rates of Production of CO_2 by Apples in Air at 65° and 38° F. after transference from 8 per cent. CO_2 at 38° F. 1947-48.

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* The ethylene is probably absorbed by the bromine, and the ethylene dibromide so formed is adsorbed by the charcoal.

the values obtained for the rate of production of ethylene in Cabinets 3 and 4 as the level for Cabinet 2, the filters on Cabinet 2 should have lasted over 100 days in 1946-7, and for a longer period than the duration of the experiment in 1947-8. Yet they began to pass ethylene when calculation shows that they could have absorbed only one third of the theoretical quantity, in 1946-7, and under one tenth in 1947-8. The reason for this is unknown.

In the cabinet fitted with the brominated carbon filter (No. 2, see Figs. 5A and 6A), the mean level of concentration of ethylene was about 2 mg. C/100 L, or about one fifth of that in the other cabinets, with minima of 0·5 and maxima of 4 to 6 mg. C/100 L, in 1946-7. In 1947-8, the mean concentration was lower, at about 1 mg. C/100 L, with maxima of 2·5 mg. and minima of about 0·6 mg., and the concentration stayed at this low level in spite of the progressive rise to about 15 to 19 mg. in the other cabinets.

The brominated carbon was a very efficient adsorbent for the " odorous " volatiles (Figs. 5 and 6, B), the level of these volatiles being lower than in the cabinet with plain carbon (No. 1). It can be seen that the mean concentration of non-ethylenic volatiles was about 0·1 to 0·2 mg. C/100 L.

5. *Condition of the apples.*

(*a*) *Superficial scald:* Scald can show itself on the King Edward VII apple in several ways. On examining a sample of these apples affected by superficial scald, and immediately on removal from storage, it is possible to separate the damaged apples into three categories, namely:

(i) Apples with a faint bronzing of the green ground colour. The lenticels tend to be more prominent, as islands of undegraded green, on these bronzed patches than on the rest of the skin.
(ii) Apples with " incipient " scald. The skin shows many light brown areas, not sunken, but with the lenticels showing as uninjured green islands.
(iii) Apples with definite sunken areas. These areas may be any colour from light to very dark brown.

There is no doubt that these types are progressively severe stages of the same disease. Microscopic examinations of cross sections through the injuries show the following:

(i) " *Bronzing* ": The epidermal cells, and occasional hypodermal cells are browned.
(ii) " *Incipient scald* ": The epidermal cells and most of the layers of the hypodermis are browned.
(iii) " *Severe scald* ": The epidermis and hypodermis are dead, and have collapsed. Some cortical parenchyma is also dead.

In all three stages, the epidermal cells around a lenticel are uninjured (in this region the hypodermis is not in contact with the epidermis).

In the 1946-7 experiments, the only injury apparent on removal of the apples from the cabinets was bronzing. After holding at ordinary temperatures for 3½ weeks, some incipient scald appeared on some of the fruit (see Table 4).* In 1947-8, all three stages of injury were present on the apples at the end of the period of gas storage (Table 5).

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* Different boxes of apples were used for the first and second examinations.

TABLE IV.

Incidence of superficial scald on King Edward VII apples, stored for 204 days in 8½ per cent. CO_2. *1946-7, 39° F.*

(*See Notes below Table IV.*)

Cabinet.	Treatment.	Scald.*		Percentage of total surface affected (incipient only).
		Percentage of apples affected.		
		Bronzed.†	Incipient.†	
First Examination. 25/4/47.				
1	Low concentration of "odorous" volatiles	6	Nil	Nil
2	Low concentration of total volatiles	3	Nil	,,
3	Oil-wrapped	Nil	Nil	,,
4	Unwrapped	1	Nil	,,
Second Examination. 19/5/47.‡				
1		3	6	1·5
2		1	7	1·5
3		Nil	8	2·0
4		Nil	18	4·5

* Mean value for six boxes per cabinet at each examination.
† Terms defined in text above.
‡ Stored at atmospheric temperatures from 25/4/47 to 19/5/47.

It will be seen from Table IV that the 1946-7 season was one in which these particular apples were not very susceptible to scald. There was no injury apparent at the first examination serious enough to reduce the market value of the apples. After being kept at ordinary temperatures they developed a small amount of scald, which was somewhat, although not significantly, more severe on the apples stored without wrappers or filters. (An analysis of the figures for the individual boxes gives $P=0{\cdot}08$, for the difference between Cabinets 1, 2, or 3, and Cabinet 4.)

Table V, which presents the results for 1947-8, shows that this was a particularly bad season for scald, some 31 per cent. of the apples stored in air, 44 per cent. of those stored in gas (unwrapped) and 10 per cent. of those in gas (wrapped) being found to be affected on removal from the cabinets. The figures for the first examination were not recorded in such a way that it is now possible to give the exact percentage of the total area scalded severely, but it was roughly half the value for incipient + severe. The severe scald doubled in amount in 11 days at ordinary temperatures. The incidence of severe scald on unwrapped apples at each examination was roughly the same whether the concentration of the odorous volatiles was allowed to remain high or was reduced (Cabinets 4 and 1 respectively). Similarly, the condition of the apples from the small gas cabinet showed that air movement within the cabinets had been without effect.

Reduction of the concentration of ethylene as well as that of the odorous fractions roughly halved the incidence of severe scald, but the amount of scald present was much greater than on the oil-wrapped apples. These could have been sold at a fair price

TABLE V.

Incidence of superficial scald on King Edward VII apples, stored for 239 days in 8½ per cent. CO_2. 1947-8. (Treatments in cabinets as in Table IV.)
(See Notes below Table V.)

Cabinet.	Mean temp. ° F.	Scald.*†		
		Percentage of apples affected by incipient + severe scald.	Percentage of total surface affected.	
			incipient + severe.	severe.
First Examination. 20/5/48.				
1	39·9	94·5	42	—
2	39·7	82·4	24	—
3	37·9	71·9	10	—
4	38·1	94·4	44	—
Small metal cabinet, conditions as 4, but no air movement	37-38	97·5	44	—
AIR	36-8	80·5	31	—
1‡	45·0	70·3	29	—
1‡	42·5	90·0	45	—
2‡	45·8	80·7	18	—
2‡	43·0	69·1	20	—
Second Examination. 31/5/48.§				
1	39·9	94·4	64	46
2	39·7	92·1	49	24
3	37·9	79·5	26	10
4	38·1	95·1	71	55

* Bronzing not counted in this season, because of extreme severity of the sunken type of scald. All cabinets contained some bronzed apples, with most in Cabinets 2 and 3, and particularly in 2.

† For Cabinets 1 to 4, values are mean of 8 boxes, except for the warm positions, where 3 to 4 boxes formed the sample.

‡ These boxes were immediately above the blowers in Cabinets 1 and 2. The temperatures are partly thermocouple records and partly flesh temperatures taken by spear thermometer when the cabinets were unloaded.

§ Stored at atmospheric temperatures from 20 to 31/5/48.

on removal from store, but the apples from the other three cabinets would have brought very low prices.

The figures for scald will be further considered in the next section.

It is not considered that the values for the warm boxes in Cabinets 1 and 2 are significantly different from those for the cooler boxes. Any slight differences cannot be attributed to temperature alone, as these warmer apples had lost more weight, those from the 45° F. boxes being severely wilted.

(*b*) *Pressure, colour, rotting, carbon dioxide injury and loss of weight:* The figures for these are given in Tables VI and VII, for the two seasons.

The final colours and pressures were similar in all cabinets, any colour difference being explicable on grounds of temperature. The apples were still hard and green when the cabinets were opened, but ripened quickly at ordinary temperatures. Any recorded differences in pressure are barely significant, but it is of interest to

TABLE VI.

Condition of apples removed from gas storage. 1946-7. (Excluding scald.)
(See Notes below Table VI.)

Cabinet.	Temperature.	Mean values.				
		Pressure. (*)	Colour. (†)	% Loss in weight.	% rotting.	% CO_2-injury.‡
Initial values 1/10/46		12·7	1·0	—	—	—
Examination I. 25/4/47.						
1	39·4	11·6	2·1	4·3	2·9	Nil
2	39·1	12·4	2·0	4·2	1·7	Nil
3	38·5	11·6	1·9	4·1	4·7	6
4	38·6	11·8	2·0	4·9	2·7	2
Examination II. 19/5/47.						
1		8·2	4·4	—	4·8	12
2		8·3	4·4	—	4·5	4
3		7·5	4·5	—	11·2	12
4		8·0	3·9	—	6·3	8

* Pressure was recorded on a peeled surface on each side of 25 apples, using a penetrometer with a $\frac{5}{16}$in. diameter plunger.

† Recorded on an arbitrary scale of coloured "ring" cards, ranging from 1—deep green, 2—yellowish green, and so on up to 5—full yellow.

‡ Carbon dioxide injury was very slight indeed, no cavitation having occurred.

note that the hardest apples were from one of the two higher temperatures, and from the cabinet with minimal concentration of ethylene, and the least hard from the coolest of all. The apples from Cabinet 2 had the least scatter of pressures, there being no apple softer than 11 lb. at the first examination, whereas there were 12 per cent. of 10 lb. readings in Cabinet 3.

The loss of weight was the same in all cabinets. The figure, 4 per cent. to 5 per cent., is surprisingly high in view of the high relative humidity maintained in the cabinets.

The unwrapped apples (Cabinets 1, 2 and 4) were less severely rotted than those in oiled paper wrappers (Cabinet 3).

CO_2-injury was slight and erratic. It did not seem to be correlated with any particular treatment. The optimum gas storage conditions for this variety are 5 to 10 per cent. CO_2, $2\frac{1}{2}$ per cent. O_2, but in this experiment 8·5 to 9 per cent. CO_2 seems to have been slightly on the high side.

In 1947-8, colours and pressures, at the first examination, appeared to be correlated solely with temperature, the hardest and greenest apples being found in the coolest cabinet. Carbon dioxide injury and low temperature breakdown were slight. Loss of weight was greatest in the warmer cabinets, but much lower than in the previous season.

Apart from scald, rotting was the only type of wastage showing interesting features. It was not related to temperature, but (compare Table V) was clearly related to the incidence of scald. In other words, the dead tissue of the scald lesions

TABLE VII.

Condition of apples removed from gas storage. 1947-8. (Excluding scald.)
(See Notes below Table VII.)

Cabinet.	Temperature.	Mean values.					
		Pressure. (*)	Colour. (†)	% loss in weight.	% rotting.	% CO_2-injury. (‡)	% break-down.
Initial values 23/9/47		12·9	1·2	—	—	—	—
Examination I. 20/5/48.							
1	39·9	9·4	2·8§	3·4	13·5	Nil	Nil
2	39·7	10·1	2·7§	3·4	6·5	5	Nil
3	37·9	10·6	2·5	1·9	6·2	4	Nil
4	38·1	10·3	2·5	2·4	19·8	7	Nil
Examination II. 31/5/48.							
1					24·8	3	3
2					12·4	Nil	2
3					7·2	Nil	Nil
4					52·2	Nil	Nil

* See note 1, Table VI.
† See note 2, Table VI.
‡ Mainly slight, but some severe with cavities; random distribution in cabinets.
§ Colour of warmer boxes: Cabinet 1: 45° F., 3·8; 42·5° F., 3·4.
Cabinet 2: 45·8° F., 4·2; 43·0° F., 3·5.

was not resistant to invasion by fungi. This is a striking demonstration of the loss of fruit, as distinct from financial loss, which may result indirectly from severe superficial scald.

DISCUSSION.

1. RESPIRATION:

The major effect of gas storage on the respiration of apples is a depression of the output of carbon dioxide, which is caused by reduction in the concentration of oxygen as well as by increase in carbon dioxide tension. This is, in fact, the aim of gas storage, since the storage life of apples is governed (in the absence of disease) by the rate of output of carbon dioxide. If the curve (Fig. 4A, a) for respiration of the apples in air is compared with the curves of Fig. 2 for similar apples in gas it is seen that the mean rate of production of carbon dioxide in air, about 75 litres/ton/day, is considerably higher than the rate in 8½ per cent. CO_2, about 45 litres/ton/day. That is, the apples in gas give off the same quantity of CO_2 in 160 to 170 days as those in air give off in 100 days. They should therefore store for about two-thirds as long again, and we know that this is about the normal extension of life caused by gas storage.

The normal course of the graph for rate of production of CO_2 by apples kept in air at a constant temperature is:

(a) An initial falling period, followed by
(b) an increase in rate (the "climacteric"),
and (c) a period of slow decline, until rotting again causes a rise.

Further, the addition of ethylene to the atmosphere in stage (a) will induce stage (b). Fig. 4A illustrates these points. The earlier climacteric of Curve (b) is probably due to accumulation of ethylene brought about by restriction of ventilation.

The normal curve for output of CO_2 in gas has not previously been fully investigated, and it is difficult to interpret the curves of Figs. 1 and 2.

Kidd and West (1934), using pre-climacteric Sturmer Pippin apples at 50° F., found that the climacteric was not inhibited by storage in artificial gas mixtures containing 5 to 13 per cent. of CO_2. It was, however, considerably delayed, relative to storage in air. It is therefore of interest to note that the occurrence of a climacteric cannot be definitely detected on the graphs for the present experiments, in which CO_2, and also the volatiles, were allowed to accumulate.

The most striking feature of the graphs of Figs. 1 and 2 is the marked peak of respiration on about the eighth day, which resembles the peak of the climacteric rise at higher temperatures of storage in air. It is probable, however, that this is not the climacteric, for the following reasons:

(1) Kidd and West (1938, a) noted a similar immediate but transitory stimulation in the respiratory activity of Bramley's Seedling apples at 10° C. (50° F.) when the air stream was changed for one containing 10 per cent. CO_2. Recovery was complete in about ten days.

(2) In the present experiments, any large increase in the concentration of CO_2 in the atmosphere, even after the apples had been in gas for many weeks, induced an immediate, transitory stimulation in the rate of respiration. This was noticed on two occasions when the suction failed, and was also induced deliberately by slowing down the aeration rate to increase the CO_2 from 8 per cent. to 10 per cent.*

(3) It was shown by Kidd and West (1938, b) that 0·1 per cent. of ethylene stimulated the respiration of pre-climacteric Bramley's Seedling apples in 10 per cent. CO_2, at 10° C. (50° F.), but once the climacteric of respiration has occurred, ethylene no longer has any effect on the rate of production of CO_2.

Fig. 1 does not reveal any difference in the rates of respiration of the apples in Cabinet 2 (with the ethylene-absorbing filter) and those in the other cabinets, but it has already been noted that the filter was not very efficient in this season (1946-7), and Curve 5 (a) shows that there was in fact a quantity of ethylene in Cabinet 2 for much of the time. The results for 1947-8 show a significant difference in the levels of respiration in Cabinets 1 and 2, which contained respectively relatively large and small concentrations of ethylene (and were at about the same temperature). Thus, from day 20 to day 90, the respiration of the apples in Cabinet 2 was the lower (the difference reaching a maximum of 20 per cent.). Griffiths and Potter (1949) have observed that King Edward VII apples, picked somewhat later from the same trees as those used in these experiments, produced more CO_2 in a gas storage atmosphere when the volatiles were allowed to accumulate than in an atmosphere swept by a rapid stream of gas of the same CO_2-content.

Thus it is probable that apples, picked before the climacteric and stored in gas in the normal way, (i.e. without removal of ethylene) have a climacteric which is masked by the fall in the rate-curve due to recovery from CO_2-stimulation. If this is so, then apples which have been gas stored for a long time, as in these tests, should not have a climacteric when transferred to air. Fig. 8 shows that when the King Edward VII apples (from Cabinet 3, 1948) were transferred to air at two temperatures (38° and 65° F.) after 239 days in 8½ per cent. CO_2, the respiration at 38° F. from the second day in air onwards, remains level and is not sensitive to ethylene. The early peak can be explained, at this temperature, by release of dissolved CO_2 from the cell sap. The course of the graph for the sample at 38° F. enables us to dismiss a climacteric rise as an explanation of the peak at 65° F. This is probably a temperature-transition effect. A. C. Hulme has found (unpublished data) that the shape of the graph for internal CO_2 is similar to that observed here for output of CO_2, when apples are transferred from air at a low temperature to air at a higher temperature.

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* This may be an important point in practical gas-storage. At some future date it will be necessary to compare the storage life of apples stored in gas mixtures, one of which is constant, and the other fluctuating widely about the same mean value.

It has been deduced that the respiration of the apples in Cabinet 2 (1947-8) was not stimulated by ethylene as early as in Cabinet 1, and it might be expected that the apples would be less yellow, and firmer, in Cabinet 2. Table VII does not show any very striking difference, but such as there is is in the expected direction. Possibly complete removal of ethylene would be profitable in giving a slight increase in storage life. It must be stressed, however, that the practical difficulty of obtaining a sufficiently fast rate of air movement from the atmosphere of the store through a filter will probably rule out any mechanical or physical method of doing this.

2. PRODUCTION OF VOLATILES:

The rates of accumulation (Figs. 5 and 6A) and of production (7A and B) of ethylene were essentially the same in Cabinets 1, 3 and 4 (i.e. those without a filter for absorbing ethylene) in any one season, but they differed in the two seasons. The graphs of Fig. 5A (1946-7) resemble in shape those obtained from a commercial store containing Cox's Orange Pippin apples (Graph E, Fig. 1, Part I of this series), in having a plateau following an initial steep rise, but those for 1947-8 are quite different from any previously obtained. In this season the production of ethylene increased at practically constant rate over the entire 239 days of gas storage. At present these differences cannot be explained, nor can any connection with the incidence of scald be deduced. All that can be said is that a higher level was reached earlier in 1946-7 than in the following season, but that the final levels were not very different.

The rate of production of ethylene in " air " (actually about 1 per cent. CO_2, Fig. 4B) was very much higher than in gas. Potter and Griffiths (1947) had observed a somewhat higher rate of production of ethylene in air than in a synthetic gas mixture containing 10 per cent. CO_2 (with, however, only 5 per cent. of oxygen), at 15° C. Further, using apples from the same source as the author, in 1947-8, they have noted that the rate of production of ethylene is lower in a " natural " gas mixture (where the CO_2 and volatiles are allowed to accumulate) than in a synthetic gas mixture, without accumulation of volatiles.

The accumulation of non-ethylenic volatiles in the two cabinets without filters (3 and 4) and the rate of production are lower than for ethylene, and the ratio of " odorous " volatiles to ethylene appears to be about the same in gas (Figs. 5 and 6, B) as in air (Fig. 4B). The rates of production are, however, higher in air.

Thus the evidence shows that gas storage reduces the actual rates of evolution of volatiles from the fruit, an effect which is probably due to the accumulation of volatiles as well as to the presence of CO_2. For the concentration of volatiles is, of course, very much higher in a gas store than in air. We do not know to what level the concentration of volatiles rises in an "air" store, but it is noteworthy that the level for odorous volatiles in the small cabinet containing about 1 per cent. of CO_2 was on the whole higher than in the cabinets (1 and 2) containing filters.

One feature of the graphs for non-ethylenic volatiles remains to be noted. In both seasons, the level of concentration (Figs. 5 and 6B) and the rates of production (Fig. 7) were initially higher for the unwrapped apples in Cabinet 4 than for those in oiled paper wrappers in Cabinet 3. This accords with the theory that the wrappers absorb volatiles. Between the 130th and 160th days the rate of production of odorous volatiles became greater in Cabinet 3 than in Cabinet 4. Either the oil-wrapped apples continued to produce odorous volatiles at an increasing rate, or the wrappers began to give off absorbed volatiles as the partial pressure of the volatiles in the atmosphere fell. Tests have shown that the oiled wraps removed from the apples in Cabinet 3 gave off non-ethylenic volatiles when aerated at about 65° F., whereas none was given off by unused wrappers. The amounts given off were 22 mg. C per kg. for the first six days, and 16 mg. C per kg. for the second 6 days. The total weight of wrappers in the cabinet might thus be capable of giving off about 350 mg. "non-ethylenic volatile" carbon when ventilated with air. The probability is high that the cross-over of the curves is due to absorption and desorption of volatiles by the oiled paper.

3. SCALD:

All the numerical experimental data have now been presented, and the more obvious deductions have been drawn. It remains to be seen whether any clear picture of the cause of superficial scald, or any ideas of how to eliminate it, can be obtained.

In 1946-7 there was practically no scald. It is, however, significant that the rate of evolution of odorous volatiles was somewhat greater than in 1947-8. Thus, if it is assumed, as it generally is, that scald is caused by some constituent of this fraction, the odorous volatiles must contain more of the toxic constituent in one season than in another, or alternatively the apple must vary in susceptibility from season to season. It is impossible to say more, in the absence of chemical analyses of the non-ethylenic volatiles. The observations do not agree with those of Smock and Southwick (1945), working with American varieties in air. They found that in two seasons when the apples developed scald, more volatiles were produced than in the season when they did not scald. It has already been mentioned in the first paper of this series that these authors use a method which involves oxidation with ceric sulphate, and that these oxidations are not stoichiometric. Thus it is doubtful if figures obtained in such a way in different seasons are strictly comparable, especially if the nature of the compounds being estimated differs.

At the outset of these trials it was expected that removal of the non-ethylenic volatiles from the atmosphere, or marked reduction in their concentration, would be accompanied by reduction in the incidence of scald, for the weight of previous evidence was in favour of the view that the odorous fractions are the causal agents. The plain charcoal filter (Cabinet 1) was reasonably efficient in absorbing the odorous volatiles, yet the amount of scald was no less than in the cabinet with the full concentration of volatiles (Cabinet 4). The level to which the concentration was reduced, namely 0·2 to 0·3 mg. C/100 L over the first 150 days, was about two thirds of the lowest mean level (not recorded in this paper) reached in the small cabinet containing apples in " air " (1 per cent. CO_2). But the apples in air were less severely scalded. If the causal agent is a constituent of the odorous fraction, then it must either be toxic in very low concentration, or it escapes estimation. It could escape estimation either by having an extremely low vapour pressure (and thus not diffusing away from the skin), or by passing through both the acid traps in the estimation train.

Attempts have been made to determine whether there is a fraction which is not trapped in the acids. For example, if a paraffinic gas were evolved, it might not be trapped in sulphuric acid. It could, however, be burnt in a furnace containing copper oxide, to yield CO_2. This CO_2 might admittedly be difficult to detect, in the presence of large quantities of CO_2 from respiration, but tests which have been carried out have not indicated that the extra CO_2 produced by burning the volatiles is greater than the amount expected from the carbon trapped in acid.

Thus the problem of the toxicity of the odorous volatiles is unresolved ; it will be returned to later.

Consider next the incidence of scald in the cabinet with the brominated charcoal filter (Cabinet 2), introduced to remove ethylene. There was very severe scald in this cabinet, but it was less than in the control cabinet. Severe scald was roughly halved by the presence of this filter. The filter had reduced the level of concentration of ethylene very markedly, and for much of the time had reduced the concentrations of odorous volatiles by about 50 per cent. below that in Cabinet 1.* Thus the reduction in scald could have been due to reduction in concentration of either of the two fractions. While it is true that Smock (loc. cit.) states that the scald produced on Rhode Island Greening apples in air by the presence of other varieties in the same store is controlled by brominated carbon and not by plain carbon, and that the same was true for McIntosh Red apples stored alone in gas, we may eliminate ethylene as the primary cause of scald for two reasons. Firstly, in Smock's experiment with McIntosh the brominated carbon would be a more efficient trap for non-ethylenic volatiles than the plain carbon, and in the experiments with Greenings it is not certain that the disease was in fact true superficial scald. It may have been confluent lenticel injury, such as has been reported by Kidd and West (1938) as being produced on English varieties, either by ethylene or by volatiles from other varieties. This type of injury would be controlled by removal of ethylene.

Secondly, the concentration of ethylene in Cabinet 3 containing oil-wrapped apples, with the lowest incidence of scald, was as high as in Cabinets 1 or 4, where scald was most severe.

The reduction in scald brought about by use of the brominated carbon filter was thus probably due to an effect on the concentration of the non-ethylenic volatiles.

Considering now Cabinet 3 (oil-wrapped, least scald), it has been shown that the concentration of odorous volatiles was higher than in the cabinets with filters, but initially somewhat lower than in the cabinet (No. 4) containing unwrapped apples with no filter.

Thus it cannot be maintained that the odorous volatiles as a whole are the cause of superficial scald. One or more of them may, however, be responsible. At present the evidence is not clear. The following theory is advanced to provide a basis for further experiments.

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* The presence of free bromine in the atmosphere was improbable. Freshly brominated carbon, aerated at a fast rate through a bed of plain active charcoal equal in thickness to that used in the upper layer of the filter, did not introduce any measurable bromine into the air stream.

4. A NEW THEORY OF THE CAUSE OF SCALD:

The main known facts about scald are as follows:

1. Scald is reduced by contact with oiled paper. This is due to removal of a factor, X.
2. Scald is reduced by warming the fruit (Kidd and West (1934)), a result discussed in detail later. This may be due to the effect of temperature on the rate of the reactions producing or eliminating factor X, or to another factor, Y.
3. Scald is not reduced by active charcoal filters, and is thus not due to volatiles generally. It is reduced somewhat by brominated charcoal filters, but this is not due to removal of ethylene.
4. A factor operative early in storage causes scald months later in storage (Kidd and West (1934)).

The new theory is that scald is caused by two factors, Y, which is volatile and X, which is not volatile.

It is an essential part of this theory that factor X is produced before factor Y. This would explain why Kidd and West (1935) found that oiled wrappers were effective early in the season in controlling later development of scald. The wrappers presumably absorbed factor X before Y was produced.

Let us consider now the explanation of the results obtained in the present experiments and by other workers, in the light of this " dual-factor " theory.

To see the picture as a whole, a third factor, not responsible for scald, must be added. This substance or group of substances, V, forms the bulk of the odorous fraction of the volatiles. It is of high volatility at 38°-40° F., whereas factor Y is presumed to have a lower vapour pressure.

In the atmosphere of a gas store, the concentrations of V and Y at the skin of the fruit will depend on, (i) the speed of flow of gas over the skin, and (ii) the concentrations of V and Y in this gas. There is a practical limit to this speed of flow; in the present experiments it was probably much higher than it is economically possible to attain in a commercial store, and thus elimination of factor Y by filters may not be practicable.

In a system containing plain activated carbon, V is almost completely adsorbed, and part of Y may be adsorbed, from the gas stream. Thus the concentration of odorous volatiles is greatly reduced. But X is still present, and Y is not wholly removed from the skin. Thus the conditions are favourable for the development of scald.

A brominated charcoal filter will also adsorb most of V, and may adsorb more of Y than plain carbon does, because Y may contain groups reactive with halogens (see later reference to Dutch work). Thus more of Y may be swept away into the gas stream, and the amount available for reaction with X will be reduced. It may in some seasons be reduced below the level of toxicity. The concentration of odorous volatiles will be somewhat lower than in the system without bromine.

Oiled wrappers may not be very retentive of the highly volatile constituent, V, and thus may produce only a slight reduction in the concentration of volatiles.

Being in contact with the skin, they could, however, absorb most of Y and possibly even some of X, and thus remove them from the zone where they cause lesions.

Kidd and West (1934) found that scald was considerably reduced on Newton Wonder apples, stored mainly at 38° F., if the apples were warmed to 60° F. for one day or to 77° F. for seven hours, at intervals of two to four weeks. This fits the theory of the presence of a substance (factor " Y ") which is not very volatile at low temperatures, but is fairly volatile (and thus escapes) at high temperature. Similarly Southwick (1945, a) reports that McIntosh Red apples gave off as great a quantity of volatiles in one day at 74° F. as in 5 months of storage at 32° F.

There is a Dutch patent (1946) for the prevention of scald by spraying apples with a solution of " Sterinobel ". This is another name for Chloramin-T, a substance which will liberate chlorine to a halogen-acceptor. This would agree with increased adsorption of factor Y by a filter containing free halogen. The actual reaction need not necessarily be halogenation, but can be an oxidation such as is produced by a hypochlorite.

The small metal cabinet used for the sample in air was sealed by vaseline, and smears of vaseline accidentally contaminated a few of the apples. The patches of skin under the vaseline were still green and uninjured at the end of the experiment, but were surrounded by a clearly delimited zone of severe scald. Thus the toxic agent or its precursors is diffusible into grease, while the vapour pressure is so low that it does not diffuse from the skin near to the vaseline spot into the vaseline. Further, the box of apples immediately above the filter in Cabinet 2 was found to be dusted with a film of fine charcoal dust, from the layer of plain charcoal on top of the filter. There was 6 per cent. of scald in this box, compared with 18 per cent. in another box, at the same temperature, which contained clean apples. Thus the carbon can adsorb factor Y, the precursor of the toxic agent, if it is brought into close proximity to it.

One of the apples in oiled wrappers was found to have an apple leaf between the skin of the fruit and the oiled paper. There was a patch of scalded tissue the size and shape of the leaf, under the leaf, surrounded by uninjured tissue. Thus the oiled wrappers are only effective if actually in contact with the skin. It is well known that shredded oiled paper does not control scald so well as oiled wrappers.

The unscalded areas of the skin are very waxy, while there is no wax on the scalded area. This may mean that the dead cells can no longer excrete wax, or that the wax acts as a natural oil-wrapper, or that the wax itself is factor X, acted on by factor Y to produce a non-waxy compound, toxic to the epidermal and hypodermal cells. Dr. E. H. Callow, who did a check analysis of the oil content of the wrappers removed from Cabinet 3, reports that the extracted oil had an iodine value of 9·7,* indicating absorption of an unsaturated compound. Dr. R. Gane has done a preliminary qualitative examination of the oil, and finds that it gives reactions for ursolic acid, a constituent of apple wax.

The wrappers from Cabinet 3 have been kept, as well as the charcoal from the filters, and these will be analysed as a first step in an investigation of the chemistry of the volatiles and waxes. It is likely that wax metabolism and volatile metabolism are linked processes. Preliminary examination of the plain charcoal has not been very successful, except in indicating that the usual method of removing adsorbed compounds from active charcoal, viz. steam distillation, cannot be applied. Ethylene dibromide has been recovered in good yield, and identified, from the used brominated charcoal.

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* The I_2-value of oil from new wrappers was 1·5.

The practical outcome of this work, to date, is to indicate that there is no immediate probability of replacing the oiled wrapper as a preventive measure against superficial scald.

5. FUTURE WORK:

The cooling and filtration systems have been rebuilt, and it is hoped to achieve uniformity of temperature between the cabinets, the number of which has been increased to ten. Future work, in addition to the chemical work already mentioned,* will include tests of other methods of absorbing volatiles, the effect of maturity of the apples, the effect of rate of accumulation of CO_2 on subsequent rate of respiration (preliminary results have already been obtained), and the effect of introducing reactive gases into the atmosphere. It is clear, however, that the problem is one which will not be elucidated in a short time.

SUMMARY.

1. King Edward VII apples have been stored in 8½ per cent. CO_2 (and 13 per cent. oxygen) at 38°-40° F. Three of the stores held unwrapped apples, and one, apples in oiled wrappers. Two of the stores of unwrapped apples were fitted with filters, which were shown by analyses of the atmosphere to reduce the concentration of volatiles. One of them removed the odorous fraction only; the other removed ethylene as well.
2. The results indicate that complete removal of volatiles from the atmosphere by means of filters, when apples are stored on a commercial scale, is not a practical possibility.
3. The considerable degree of reduction in concentration of the odorous volatiles, brought about by the filter, did not result in any control of superficial scald. (Scald in filtered gas, 42 per cent.; in control, 44 per cent.)
4. The somewhat more efficient removal of odorous volatiles, accompanied by a reasonably efficient removal of ethylene, did give a certain measure of control of scald. (Scald in filtered gas, 24 per cent.; in control, 44 per cent.)
5. Oiled wrappers were more efficient than filters in preventing scald and have no immediate substitute for this purpose. (Scald in oiled wrappers, 10 per cent.; in control, 44 per cent.)
6. The conclusion is drawn that one of the causal factors of scald is a substance of very low volatility at the temperature of storage, which can only be removed by agents in contact with or very close to the skin. (Factor Y.)
7. A new theory is advanced, namely that scald can only occur in the presence of Factor Y which is volatile, and another Factor, X, which is not volatile.
8. The immediate effect of high concentrations of CO_2 in the gas-storage of apples is to induce a transitory rise in the rate of respiration. Following this, it depresses respiratory activity below the level normal for respiration in air.
9. Apples in gas-stores with normal accumulation of volatiles undergo a climacteric rise in respiration.

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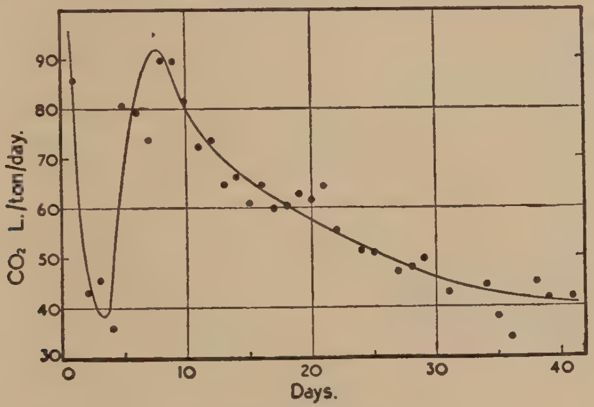
* In order to avoid spurious results due to odours given off by the wood of the boxes, metal boxes will be used in future.

10. The climacteric is delayed if the concentration of ethylene in the atmosphere is considerably reduced.

Thanks are due to my colleagues, mentioned in this paper, for assistance and for provision of unpublished data, also to Mr. C. J. North, who did most of the analyses, and to Mr. G. W. Goodwin and Mr. C. W. Croxon who were responsible for the engineering and for temperature measurements.
This work formed part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

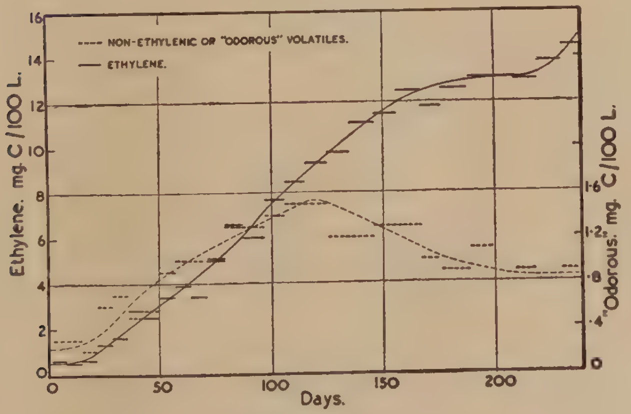
APPENDIX.

Graphs A and B are examples of the actual values found in typical experiments, for comparison with the smoothed curves given in this paper.



APPENDIX FIG. A.

Rate of Production of CO_2. 1947-48.
Cabinet No. 2. Days 0-40. Actual observed values.



APPENDIX FIG. B.

Concentration of Volatiles. 1947-48.
Cabinet No. 4. Actual observed values.

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(*Received* 7/1/49.)

FURTHER EXPERIMENTS ON THE CONTROL OF THE APPLE BLOSSOM WEEVIL, *ANTHONOMUS POMORUM* L.

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It has been shown in a previous paper (Dicker, Gayner and Austin, 1946) that excellent control of the Apple Blossom Weevil could be obtained when 5 per cent. DDT dust was applied at the bud burst stage and again a week later. There were also indications that a 5 per cent. benzene hexachloride dust, similarly applied, might prove effective and promising results had been obtained in laboratory trials with DDT sprays. Further field trials have since been carried out at East Malling to discover whether one application of dust would give adequate control and to determine the minimum concentration of DDT necessary in a spray.

Fairly critical timing was essential when using dusts and the spray application meant an addition to the routine spray schedule; hence efforts were also directed towards the possibility of incorporating DDT in the petroleum or DNC-petroleum sprays, which are normally applied from two to five weeks before the bud burst stage, in the hope that the DDT deposit would remain toxic until all adults had emerged from hibernation. Such good results were obtained that the trials were extended to investigate the possibilities of adding DDT to the tar oil spray, mainly to benefit growers of cider apples and gardeners who use only this type of spray during the dormant period.

Results have confirmed that DDT persists in toxic form for many weeks and a variety of measures are now available to the fruit grower for the control of this pest, varying from DDT in a late tar oil spray to the application of a DDT dust or spray at the bud burst stage.

DUSTING TRIALS, 1946.

The purpose of these trials was to compare the effectiveness of one and of two applications of dusts containing 5 per cent. of DDT and benzene hexachloride (BHC) respectively. As it was impossible to find a sufficient acreage of uniformly infested apples to combine all the treatments in one trial and also leave adequate guard rows to allow for drift, the BHC trial was carried out at the Research Station and the DDT dust applied on a neighbouring farm.

A double row of the variety Lane's Prince Albert was used for the BHC trial, the rows running from north to south. This type of elongated, narrow block had proved very satisfactory for dusting trials as, with the prevailing wind coming from a westerly direction, there was practically no risk of the dusts drifting on to trees receiving other treatments. The trees were divided into seven plots, each consisting of a double row-length of eleven trees. Each treatment was duplicated, with three controls. The BHC, of technical grade containing approximately 10 per cent. gamma isomer, was diluted with a carrier composed of gypsum and china clay and applied with a hand duster of the rotary type at the rate of 45 lb. per acre on 30th March and 4th April. As the trees were small, results were assessed by counting the number of capped blossoms in a single sample of 200 trusses from each of ten trees in the centre of each plot.

The DDT trial was carried out in an orchard planted with the varieties Bramley, Grenadier and Lord Derby. It was rectangular in shape and divided into seven plots of approximately half an acre each, running the width of the orchard. Again the treatments were duplicated, with three controls. The DDT, of technical grade containing 80 per cent. *p-p'* isomer, was diluted with china clay. It was applied with a power duster at the rate of 40 lb. per acre on 29th March and 4th April.

TABLE I.

Dusting Trials, 1946.

Treatment.	Dates of application.	Mean % capped blossoms.	% reduction.
5% DDT	29/3/46	1·4*	90·8
5% DDT	29/3/46 4/4/46	0·9*	94·1
Control	—	15·3	—
5% BHC	30/3/46	26·9	17·5
5% BHC	30/3/46 4/4/46	11·2*	65·6
Control	—	32·6	—

* Indicates significance.

From each of six trees of the variety Grenadier, situated near the centre of each plot, the number of capped blossoms was counted in 200 trusses. These trees were much larger than in the BHC trial and, though previous work (Dicker, 1946) had shown there was no difference in infestation due to a positional effect on the tree, any possible source of error due to sampling was minimized by taking 50 trusses from the north, east, south and west side of each tree.

In each trial the fruit buds varied between the bursting and burst stages when the first dust was applied. The weather was hot and sunny and, except for 0·3 in. on 4th and 5th April, no rain fell until 17th April. The majority of the weevils arrived on the trees from hibernating quarters during the last week in March, emergence being complete by 8th April. The first egg was found on 29th March.

Although the dusts were applied by different methods, a good cover was always obtained and it is improbable that method of application has influenced the results. The method of application may have slightly favoured the BHC as, by hand dusting smaller trees, a more uniform cover was possible than where DDT was applied with a power duster.

The results, presented in Table I, show that a single application of 5 per cent. DDT dust at the bud burst stage reduced the number of capped blossoms by 91 per cent., a second application six days later giving only slightly better control. Whilst there is no significant difference between treatments, both differ in a highly significant way from the control. These figures indicate that an adequate commercial control of the Apple Blossom Weevil can be obtained by dusting once, at the bud burst stage, with 5 per cent. DDT.

With BHC, on the other hand, the first dust only slightly reduced the infestation, whilst two applications gave significantly better control, amounting to a 66 per cent. reduction in capped blossoms. These results confirm the conclusions drawn from previous experiments, namely, that at the same concentration a DDT dust is more effective than one containing BHC.

SPRAYING TRIALS, 1946-48.

(*a*) *Sprays containing DDT alone and mixed with petroleum.*

In 1946 and 1947, experiments, detailed in Table II, were carried out in an orchard planted in 1935 with bush trees of the varieties Cox and Worcester, there being three rows of Cox alternating with each row of Worcester. Each treatment, randomized and replicated four times, was applied to a square plot of 16 trees so arranged that the Worcesters always formed an outside row. The DDT was of technical grade containing 82 per cent. *p-p'* isomer. For straight sprays the DDT was dissolved in benzene and emulsified with the anhydrous *cyclo*hexylamine salt of Sulphonated Lorol; when mixed with petroleum it was added to a proprietary concentrate of the miscible type a week previous to application and stirred frequently until dissolved.

Each year the entire orchard received a routine 5 per cent. tar oil wash in January and a 6½ per cent. petroleum spray was applied in early March to all plots except those receiving DDT mixed with petroleum. A canvas screen was used to prevent drift when the experimental sprays were applied. Results were assessed from the four centre trees of each plot by counting the number of capped blossoms in samples of 200 trusses distributed equally on the north, east, south and west sides of each tree.

As the level of infestation in 1947 was low, another site was chosen for the 1948 trial (Table III). This consisted of a double row of the variety Lane's Prince Albert.

TABLE II.

Field spraying trials, 1946-47, using DDT alone and mixed with petroleum.

Treatment.	1946			1947		
	Date applied.	Mean % capped blossoms.	% reduction.	Date applied.	Mean % capped blossoms.	% reduction.
0·025% DDT +6½% petroleum	—	—	—	March 17th	1·9	72
0·05% DDT +6½% petroleum	March 23rd	0·3	97	,	1·1	84
0·1% DDT +6½% petroleum	,,	0·2	98	,,	0·3	96
0·2% DDT +6½% petroleum	,,	0·1	99	—	—	—
0·025% DDT	—	—	—	March 17th	9·2	—35
0·05% DDT	March 23rd	2·6	73	,,	5·5	19
0·1% DDT	,,	0·7	93	,,	1·0	90
0·2% DDT	,,	0·3	97	—	—	—
0·0125% DDT	—	—	—	April 1st	2·3	66
0·025% DDT	March 30th	2·8	71	,,	2·4	65
0·05% DDT	,,	1·7	83	,,	3·1	54
0·1% DDT	,,	1·5	85	—	—	—
Control	—	9·	—	—	6·8	—

Each plot occupied a double row-length of eight trees, the treatments being randomized and replicated three times. Records were taken as before from the eight central trees of each plot.

Biological observations indicated that the majority of weevils arrived on the trees during the periods 21st March-2nd April, 25th March-10th April, and 8th-24th March in 1946, 1947 and 1948 respectively. The first eggs were found on 28th March, 1946, 12th April, 1947, and 25th March, 1948.

TABLE III.

Field spraying trial with DDT, 1948.

Treatment.	Date applied.	Mean % capped blossoms.	% reduction.
0·0125% DDT	March 23rd	2·3	94
0·025% DDT	,,	0·6	98
0·05% DDT	,,	0·2	99
Control	—	36·5	—

Analysis of the results, summarized in Table II, showed that in 1946 all DDT treatments were significantly better than the controls ; in 1947, for reasons to be explained later, only the results of treatments applied on 17th March were analysed and, with the exception of 0·025 and 0·05 per cent. DDT, these also differed significantly. Also, for sprays applied at the same time, on 23rd March, 1946, and 17th March, 1947, DDT mixed with petroleum gave significantly better control than DDT alone.

In 1946, when used with petroleum, all concentrations gave a very high degree of control ; when applied as straight DDT sprays on the same date the lowest concentration, 0·05 per cent., was significantly less effective than 0·1 or 0·2 per cent. A slightly lower range of concentrations tested in 1947 again resulted in all the petroleum combinations giving good control, none of which differed significantly, whilst for DDT alone only 0·1 per cent. was effective. It will be noticed that for any given strength the degree of control was less in 1947 than the previous year. This can be explained by the fact that in 1946 the interval between spraying and the onset of egg laying, which in most years follows a few days after the peak emergence from hibernation, was only five days, whereas in 1947 the first egg was not found until 12th April, 26 days after the sprays were applied. Even after this interval 0·05 per cent. DDT in petroleum reduced the infestation by 84 per cent., which on a commercial scale would be considered quite satisfactory. Whilst 0·1 per cent. DDT gave good control in both years, the 0·05 per cent. spray reduced capped blossoms by only 73 per cent. in 1946, an indication that its residual toxicity did not persist until the end of the first week in April by which time all weevils had emerged from hibernation ; in 1947 0·05 per cent. was almost ineffective when applied nearly four weeks before egg laying began.

The results obtained with straight DDT sprays applied later, on 30th March, 1946 and 1st April, 1947, are not so consistent. In the first place, application of the spray in 1946 was unavoidably delayed until a few days after egg laying had begun. It will be noticed that 0·1 per cent. DDT applied on 23rd March, 1946, reduced the capped blossoms by 93 per cent., whilst for a spray of the same strength applied a week later the figure was only 85 per cent. This is due to the latter spray being applied after some eggs had already been laid in the blossom buds, these being unaffected by the spray which was directed towards killing the adults before oviposition commenced. In spite of these unfavourable conditions the infestation was reduced by 83 per cent. with 0·05 per cent. DDT and, had these sprays been applied a few days earlier as was originally intended, it is considered that much better control would have been obtained. The fact that only a very slight increase in control, from 83 to 85 per cent., was noted when the concentration of DDT was increased from 0·05 to 0·1 per cent., lends further support to this assumption ; for it suggests that these two concentrations were giving almost complete control after the time of application, the recorded infestation being due almost entirely to eggs laid before the sprays were applied.

Something obviously went wrong with the DDT sprays applied on 1st April, 1947. All three concentrations gave approximately the same degree of partial control, there even being a suggestion of reduced effect with increasing concentration of DDT. This points to an error in formulation or mixing in the spray tank which it has been found impossible to explain. No conclusions can be drawn from these results.

In the 1948 trial, Table III, the spray was correctly timed, going on two days before any eggs were found. The results are far superior to those obtained in 1946 and indicate that a strength as low as 0·025 per cent. will give almost complete control of the Apple Blossom Weevil. When the 1946 and 1948 results are considered together and due allowance is made for the unavoidable lateness of application in 1946 the author considers a very satisfactory measure of control can be obtained by spraying with 0·025 per cent. DDT just before egg laying begins, i.e. at the bud burst stage.

One difficulty was encountered in connection with the rather elaborate layout of the 1946 and 1947 trials. In order to provide adequate replication the plots had to be small, each consisting of only 16 trees, and this did not allow sufficient room for normal movement of the weevils within the plot. In practice a large proportion of the adult weevils hibernate in cracked fencing posts and other suitable habitats around the edges of orchards and, following emergence from hibernating quarters, there is a considerable movement from tree to tree which continues throughout the pre-maturation feeding and oviposition periods. Now, in both years the first DDT sprays were applied before many weevils had arrived on the trees and in the course of the general dispersal which followed many alighted on sprayed trees and were killed before the normal distribution of weevils within the orchard was complete. After all the weevils had returned from hibernation the only populated plots would be the controls and those sprayed with sub-lethal concentrations of DDT. With such small plots as used in these experiments a movement of weevils from plot to plot will still occur and should any alight on trees sprayed with lethal concentrations of DDT before oviposition is complete they will be killed, still further depressing the infestation. These effects could be traced with certainty on the control plots where the percentage capped blossoms within the treated block was only half that on an adjoining portion receiving no DDT. This difference was not a positional effect.

(*b*) *Sprays containing DDT mixed with DNC-petroleum.*

As a result of the very effective control obtained when DDT was added to petroleum it was considered that similar results should be obtained by incorporating DDT in the combined DNC-petroleum wash, often used commercially as an alternative to the tar oil and petroleum sprays. To check this assumption a trial was laid down in 1947. A plot of the variety Lane's Prince Albert from which alternate trees had been removed in every other row was chosen for the experiment. Two adjacent rows were selected to receive the experimental sprays, each treatment being applied to a triangular plot containing three trees and replicated four times. A corresponding pair of rows separated from the treated trees by a single row was similarly subdivided and served as controls. Thus every treated plot had its corresponding control plot. Contamination of neighbouring plots by spray drift was prevented by using a canvas screen. The DDT, from the same batch and emulsified in the same way as in previous experiments, was added to the spray tank to give concentrations of 0·05, 0·1 and 0·2 per cent. respectively after the DNC-petroleum had been diluted with water. The DNC-petroleum, a commercial product, was used at 8 per cent. which gave approximately 0·1 per cent. DNC and 6 per cent. petroleum in the dilute wash. The sprays were applied on 3rd March, the only suitable day in a period of very hard weather extending from the end of January to mid-March. Conditions were sufficiently favourable for the spray deposit to dry on all trees before the afternoon temperature dropped to freezing point. During the following fourteen days the minimum temperature recorded in the screen was below 32° F. on eleven occasions, the lowest being 22° F. on 8th March. Results were assessed by counting the number of capped blossoms in 400 trusses, subdivided into samples of 100 trusses from the north, east, south and west side, from each sprayed tree.

As will be seen from Table IV, good control was obtained with all concentrations of DDT used. Capped blossoms were reduced by 88 and 97 per cent. with sprays containing 0·05 and 0·1 per cent. DDT respectively, but no further increase in control was obtained by raising the DDT concentration to 0·2 per cent. Statistical analysis showed the 0·1 and 0·2 per cent. treatments to be significantly better than the 0·05 per cent. spray.

The type of layout employed, namely a double row of trees in the midst of an otherwise untreated plot, meant that the DDT sprays underwent a much more severe test than would normally occur under commercial conditions where the entire orchard would be sprayed. For not only did the DDT have to remain toxic until all weevils had emerged from hibernation, but the treated plots were also subject to reinfestation from neighbouring unsprayed trees throughout the oviposition period, it being well established that adult weevils move from tree to tree throughout this period. Egg laying did not begin until 12th April, 40 days after the sprays were applied, and oviposition continued for a further two to three weeks. It would appear, therefore, that at 0·05 per cent. the DDT deposit was beginning to lose its toxicity after a period of six or seven weeks whilst the 0·1 per cent. spray was still effective at the end of eight weeks. It is also of interest to note that the relatively low temperatures experienced in the days immediately following application apparently had no effect on the insecticidal value of the spray residue.

(*c*) *Sprays containing DDT mixed with tar oil.*

On 11th February, 1946, a small scale trial was carried out by spraying single

TABLE IV.

Field spraying trial, 1947, using DDT mixed with DNC-petroleum.

Treatment.	Date applied.	Mean % capped blossoms.	% reduction.
0·05% DDT + 8% DNC-petroleum	March 3rd	2·4	88
0·1% DDT + 8% DNC-petroleum	,,	0·6	97
0·2% DDT + 8% DNC-petroleum	,,	0·6	97
Control (8% DNC-petroleum)	,,	19·9	—

trees with DDT dissolved in tar oil and also as an acetone-carbitol emulsion added to the diluted wash. With a concentration of 0·2 per cent. DDT in 5 per cent. tar oil both formulations reduced the percentage capped blossoms from 28·5 to 3·0, indicating there was no significant difference in toxicity whether the DDT was present in the oil or water phase. In subsequent trials technical grade DDT, from the same batch as used in previous experiments, was added to the spray concentrate several days prior to application and stirred frequently until dissolved, or a wettable powder containing 20 per cent. DDT precipitated on china clay with sulphite lye as a wetting agent was added to the spray tank after the tar oil had been diluted. In all experiments the tar oil was used at 5 per cent. of the spray concentrate, giving approximately 3 per cent. neutral oil in the dilute wash.

The 1947 trial was carried out in an orchard of mixed apples and pears, the apple variety Lane's Prince Albert predominating. Each treatment was replicated three times, the plot size being one-seventh of an acre. Due to a prolonged period of arctic weather, spraying was delayed until 12th March when the control plots and those receiving 0·05 per cent. DDT were sprayed. The trees dried very slowly and before the 0·05 per cent. application had been completed a light drizzle set in, turning to steady rain later in the evening; the temperature also dropped and slight freezing

TABLE V.

Field spraying trials, 1947-48, using DDT mixed with 5 per cent. tar oil.

Treatment.	1947			1948		
	Date applied.	Mean % capped blossoms.	% reduction.	Date applied.	Mean % capped blossoms.	% reduction.
0·05% DDT dissolved in tar oil	March 12th	4·9	46	—	—	—
0·1% DDT dissolved in tar oil	March 15th	0·9	90	Feb. 11th	0·4	95
0·2% DDT dissolved in tar oil	—	—	—	,,	0·1	99
0·1% DDT wettable powder + tar oil	—	—	—	,,	1·1	86
0·2% DDT wettable powder + tar oil	—	—	—	,,	0·3	96
Control (tar oil)	March 12th	9·1	—	,,	7·6	—

of the spray deposit was observed on the branches. The 0·1 per cent. DDT went on under favourable conditions on 15th March. Results were assessed by counting the number of capped blossoms in 400 trusses, subdivided into samples of 100 trusses from the north, east, south and west side, from each of four trees of the variety Lane's situated near the centre of each plot.

The results, presented in Table V, show that a good control, amounting to approximately 90 per cent. reduction in capped blossoms, was obtained with 0·1 per cent. DDT, whilst at 0·05 per cent. the infestation was not quite halved. There were indications that the efficiency of the 0·05 per cent. spray was reduced due to unfavourable weather conditions immediately following the spray application, for the infestation was considerably lower on the first plot sprayed, where the spray deposit had time to dry before rain set in, than on the other two plots.

The 1948 trial was carried out on the same trees as used in 1947 for the DDT-DNC-petroleum sprays, the layout being identical except that each treatment was replicated three times. The sprays were applied under ideal conditions on 11th February. As will be seen from Table V all treatments gave a highly significant degree of control. Though no significant differences could be detected between treatments there was a suggestion that DDT dissolved in tar oil was slightly more toxic than a DDT wettable powder.

Egg laying did not begin until 25th March, 43 days after spraying, and continued for nearly three weeks, hence DDT at 0·1 per cent. when dissolved in tar oil and at 0·2 per cent. as a wettable powder gave almost complete protection against the Apple Blossom Weevil for a period of at least eight weeks. The slightly lower control obtained with DDT at 0·1 per cent. in wettable powder form suggests this spray was beginning to lose its toxicity after six or seven weeks.

OBSERVATIONS FOLLOWING ROUTINE FARM SPRAYS

Fruit growers began using DDT in the routine DNC-petroleum or petroleum sprays against the Apple Blossom Weevil in 1947 and reported excellent control. In 1948, with the exception of three experimental plots, all apples on the Research Station received DDT in one of the above sprays. It was applied by the usual spray gang with no more supervision than is required for normal commercial spraying. Subsequently counts were made on five plots which in previous years had been regularly infested with the Apple Blossom Weevil. Though no detailed records

TABLE VI.

Results obtained with routine farm sprays, 1948.

Plot.	Variety.	Treatment.	Date applied.	No. of capped blossoms in 2,400 trusses.
35	Cox	0·05% DDT + 4% DNC-petroleum	March 1st	0
K7	Worcester	,, ,,	,, 3rd	1
11	Lane's	,, ,,	,, 8th	14
M2	Cox	,, ,,	,, 13th	1
C3	Lane's	0·05% DDT + 4% petroleum	,, 10th	0

were available it can safely be assumed that on these plots the annual infestation prior to 1948 had averaged 10-20 per cent. The DDT used was a commercial preparation in wettable powder form, containing 20 per cent. DDT. Twelve trees, evenly distributed throughout each plot, were selected for records and the number of capped blossoms counted in 200 trusses, subdivided into samples of 50 trusses from the north, east, south and west side, from each tree. Each truss carried approximately five blossoms. It will be seen from Table VI that for all practical purposes a complete control of the Apple Blossom Weevil was obtained.

DISCUSSION OF RESULTS OBTAINED IN SPRAYING TRIALS.

Whilst satisfactory control was obtained with all types of spray used in these experiments the outstanding feature has been the marked effect of incorporating DDT in an oil spray. For instance, in the 1946 and 1947 trials (Table II) where a direct comparison was made between straight DDT sprays and DDT dissolved in petroleum, all concentrations gave significantly greater control when mixed with oil. In 1946 this was especially noticeable with the 0·05 per cent. DDT spray applied on 23rd March, the percentage reduction in capped blossoms being 73 and 97 for straight and oil sprays respectively. The increase was still more spectacular for sprays applied on 17th March, 1947, the percentage reduction rising from —35 and 19 to 72 and 84 for 0·025 and 0·05 per cent. DDT respectively. The increased efficacy of DDT in petroleum over a straight spray can probably be attributed to the physical condition of the deposit and the extra protection afforded by the oil film in reducing the effect of weathering on the spray deposit.

Similar persistent effects were noticed when DDT was combined with DNC-petroleum and tar oil and, though no sprays containing DDT alone were applied for comparison, it seems safe to assume that both prolonged the residual toxicity. The results suggest that, against the Apple Blossom Weevil, DDT persists in toxic form for even longer periods when applied with DNC-petroleum and tar oil than in petroleum. It must, however, be borne in mind that these experiments were spread over a period of three years and the variable conditions under which the trials were carried out may equally well provide the explanation of these differences.

In calculating the residual toxicity of the DDT deposit it must be remembered that whereas the intervals quoted relate to the period between the date of application and the discovery of the first egg, due allowance must also be made for the fact that weevils continue to emerge from hibernation for at least a week after egg laying begins ; also, under the conditions prevailing in these experiments the treated plots were subject to reinfestation by adults from control plots or other neighbouring untreated trees throughout the oviposition period which lasted for approximately three weeks.

DDT at a concentration of 0·05 per cent. when dissolved in petroleum reduced capped blossoms by 97 per cent. in 1946 and 84 per cent. in 1947, the intervals between spraying and the discovery of the first egg being 5 and 26 days respectively. In the 1947 trial where 0·05 per cent. DDT emulsion was added to a DNC-petroleum spray, applied 40 days before egg laying began, capped blossoms were reduced by 88 per cent. Thus the effective duration of the DDT deposit at 0·05 per cent. when combined with petroleum or DNC-petroleum is not less than four weeks. This was confirmed by the results of commercial spraying at the Research Station in 1948 when virtually complete control of the Apple Blossom Weevil was obtained with sprays applied up to 24 days before oviposition began. In this case whole orchards were sprayed and therefore no reinfestation was likely to occur from unsprayed trees but the deposit must have remained toxic for a sufficient period after oviposition began to kill those weevils emerging late from hibernation. The maximum effective period for 0·1 per cent. DDT dissolved in oil has not been accurately determined as, in all experiments with the exception of the 1947 tar oil trial (Table V), at least 95 per cent. reduction in capped blossoms was obtained. Under the conditions of these experiments it is estimated that 0·1 per cent. DDT remains effective for at least eight weeks. A DDT wettable powder was used only once, in the 1948 tar oil trial (Table V), where at 0·1 per cent. it appeared to be losing its toxicity after six or seven weeks.

Biological observations on the Apple Blossom Weevil have been made regularly since 1943 and in four of these years the first egg was found during the period 25th March-3rd April. The two exceptions are 17th March, 1945, an exceptionally early season, and 12th April, 1947, following an intensely cold spell which extended from late January to mid-March. Thus, in the average season, egg laying can be expected to begin at the end of March.

The application of these results to commercial spraying means that the former recommendation of dusting at the bud burst stage will now be superseded by the inclusion of DDT in one of the winter washes. Normally it will be added to the petroleum or DNC-petroleum spray and for an average season the author considers 0·1 per cent. DDT should be used if these sprays are applied in February, whilst a March application will need only 0·05 per cent. DDT. For those who use only tar oil the application needs to be delayed until February and 0·1 per cent. DDT added.

Observations are being continued on the Research Station to study the rate of reinfestation following the almost complete destruction of this pest, as occurred in 1948. It is obvious that an annual spray will be unnecessary—in all probability three or four years will elapse before the Apple Blossom Weevil is again sufficiently numerous to merit any control measures being taken.

SUMMARY.

During the period 1946-8 a number of dusting and spraying trials were carried out against the Apple Blossom Weevil, *Anthonomus pomorum* L. One application of 5 per cent. DDT dust, applied at the bud burst stage, reduced capped blossoms by 91 per cent. and a second applied six days later gave only slightly increased control. Under similar conditions a 5 per cent. benzene hexachloride dust reduced capped blossoms by 18 and 66 per cent. respectively. Effective control was also obtained by spraying with 0·025 per cent. DDT at the bud burst stage. From other experiments it is concluded that good control will be obtained with 0·05 or 0·1 per cent. DDT in petroleum or DNC-petroleum according to whether the application is made in March or February and 0·1 per cent. DDT applied in tar oil during February.

The residual toxicity of the various sprays is discussed and it is shown that, when applied in oil, the persistence of the DDT deposit is increased.

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MOSAIC DISEASE OF THE RASPBERRY IN GREAT BRITAIN

III. FURTHER EXPERIMENTS IN SYMPTOM ANALYSIS

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In experiments on the transmission of raspberry virus diseases, Harris (1940) showed that the raspberry variety Baumforth's Seedling B is valuable as an indicator since it has a distinctive reaction to each of two disease groups, Mosaic 1 and Mosaic 2. He also obtained evidence that the variety Lloyd George does not show symptoms when infected with Mosaic 2. The present paper describes experiments to determine the reaction of a number of varieties to infection with Mosaic 2 and to obtain information on the state of health of existing commercial stocks.

LLOYD GEORGE.

In 1943 a number of grafts were made to investigate and compare the virus status of three stocks of Lloyd George (A, B and C), inter-related as follows. A and B were derived directly from the same visually mosaic-free selection made at East Malling in 1927 but, whereas B had been propagated continuously at East Malling, A had been transferred in 1930 to a farm isolated from other raspberries, where it has been propagated on a commercial scale. Stock C was derived from a re-selection from stock B on the basis of graft tests to Baumforth B indicators in 1939. All three stocks had been carefully rogued for visual symptoms of mosaic. Sample A had maintained to a high degree the vigour characteristic of the variety, but B and C were only moderately vigorous at the time of the tests. None of the plants under test showed symptoms of mosaic infection but plants of samples B and C showed slight yellowing of the smaller leaf-veins and a faint leaf mottle. Ten plants of each of the three selections were grafted to Baumforth B plants by the cane-inarching method (Harris, 1940) and ten ungrafted Baumforth B plants served as controls.

In 1944 all ten of the Baumforth B (indicator) plants grafted to plants of sample A developed symptoms of Severe Mosaic 2. Since the Lloyd George plants remained normal in appearance it was thus confirmed that Lloyd George does not show diagnostic symptoms when infected with Severe Mosaic 2 alone.

The indicators grafted to plants of the other samples developed symptoms of a previously unrecognized type. In 1944 the canes on the infected indicator plants were rather more numerous than on the controls, the internodes were shortened and the plants slightly reduced in vigour. The most conspicuous symptom, however, was a downward curling of the leaves (Figs. 1 and 3). This leaf-curling varied in intensity, being severe in some plants and quite slight in others. As, however, uninfected Baumforth B plants tend to produce slightly curled leaves, positive identification of infection was sometimes difficult.

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In the following year (1945) symptoms were more conspicuous. Numerous very weak, short canes were produced on infected plants and the leaves were down-curled (Fig. 2).

The name " Curly Dwarf " is provisionally proposed for this disease on Baumforth B and the " Raspberry Curly Dwarf Virus " for the virus producing the symptoms. It is probable that the faint mottling and vein yellowing visible on the Lloyd George plants of samples B and C were produced by the Curly Dwarf virus, but as virus-free plants of the variety were not available it was not possible to confirm this by re-infection.

The results of these experiments and of a number of others with samples of different selections of Lloyd George are shown in the Table.

TABLE I.

Graft tests of Lloyd George samples.

Year.	Lloyd George Sample.	No. of plants.	Symptoms on Baumforth B indicators.				
			Dead.	Severe Mosaic 2.	Curly Dwarf.	Other symptoms.	No symptoms.
1943	A	10	—	10	—	—	—
	B	10	1	—	9	—	—
	C	10	—	—	10	—	—
	Ungrafted control	10	—	—	—	—	10
	D	3	1	—	2	—	—
1945	A	5	1	3	—	1*	—
	E	10	—	9	—	1†	—
	F	10	1	9	—	—	—
	Control	20	—	—	—	—	20
1946	G	20	—	17	1	1*	1
	Control	10	—	—	1	—	9

* Indeterminate. † Mild Mosaic 2.

It will be seen that the majority of the plants included in these samples were infected with Severe Mosaic 2 or with Curly Dwarf. The effect of Severe Mosaic 2 on Baumforth B is so drastic that it was not found possible to diagnose joint infections with Severe Mosaic 2 and Curly Dwarf with any certainty. It is probable, however, that many of the plants which produced symptoms diagnosed as Severe Mosaic 2 were infected also with Curly Dwarf. The single plant of sample G which produced no symptoms on the indicator was itself not vigorous and it is unlikely that it was in fact virus-free.

NORFOLK GIANT.

In the field, naturally-infected plants of this variety show symptoms of several distinct types and it therefore seemed that it might prove a useful indicator to supplement or replace Baumforth B which is less vigorous and is now only rarely grown commercially.

In 1944, therefore, five plants of one symptomless (mosaic-rogued) selection of this variety (L.209) and ten plants of another similar selection (L.234) were grafted to Baumforth B indicators. Four plants of each selection grafted in pairs, and ten ungrafted plants of Baumforth B, served as controls. No symptoms developed on any of the control plants or on the Baumforth B indicators.

One of the L.209 plants that had been grafted to Baumforth B died. The remaining four developed no symptoms and four of the L.234 plants developed no symptoms. The other six L.234 plants grafted to Baumforth B developed slight vein chlorosis on small areas of some of the leaves. These symptoms are thought to have been caused by virus infection but the origin of the infection is not clear. It is possible that the plants were already infected when selected in 1944, but if so it is remarkable that none of the control Norfolk Giant plants developed symptoms. Alternatively, the symptoms may have been caused by infection with an unsuspected virus present in the Baumforth B plants.

Five plants of each selection of Norfolk Giant were grafted to Red Cross plants infected with Severe Mosaic 2. All of the Norfolk Giant plants developed intense symptoms. The plants were stunted and the leaves distorted with depressed, sharply-defined chlorotic areas (Fig. 4). Symptoms of Mosaic 2 on Norfolk Giant are thus similar to those on Baumforth B (Harris, 1933).

In 1944, ten Norfolk Giant plants were grafted to Lloyd George plants showing symptoms of mosaic (caused by joint infection with Mosaic 1 and Mosaic 2—Harris, 1940). One graft failed and the other nine Norfolk Giant plants developed symptoms of Severe Mosaic 2. Owing to the severity of the symptoms it was impossible to diagnose any symptoms which may have been produced on the indicators by Mosaic 1.

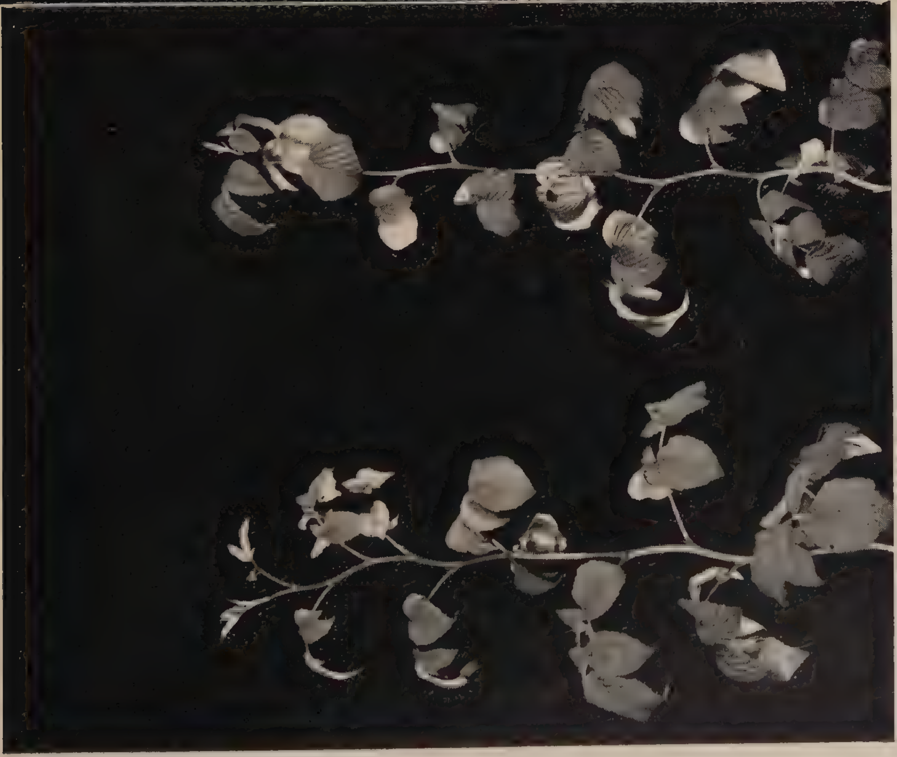
In 1945, five Norfolk Giant plants were grafted to Lloyd George plants showing no symptoms (Selection A). Four of the Norfolk Giant plants developed symptoms of Severe Mosaic 2.

As a result of these various tests it is concluded that Norfolk Giant plants showing no symptoms produce no symptoms on Baumforth B and may therefore be presumed to be virus-free. It is also concluded that Norfolk Giant is a sensitive indicator of Severe Mosaic 2 and may be useful to supplement or replace Baumforth B.

MALLING LANDMARK.

In 1942, 10 plants of the variety Malling Landmark, showing no symptoms except a very faint mosaic mottle which appears to be characteristic of the variety, were grafted to Baumforth B indicators. No symptoms were produced on the indicators and it was concluded that the sample of the variety was virus-free. Malling Seedling " B " (a variety which previous graft test had shown to be infected with Severe Mosaic 2), Malling Landmark and Baumforth B were combined in graft units of three plants as follows : Landmark grafted to Malling B grafted to Baumforth B (5 sets) ; Landmark grafted to Malling B grafted to Landmark (5 sets). None of the 15 Landmark plants developed symptoms and each of the 5 Baumforth B plants developed symptoms of Severe Mosaic 2. As a further test of the virus content of Malling B, 5 plants were grafted to Baumforth B. All 5 Baumforth B plants developed Severe Mosaic 2 symptoms.

To confirm that the Landmark plants were actually infected although showing no symptoms, 12 of these plants were regrafted to Baumforth B indicators in 1943. One of the indicators died, ten developed symptoms of Severe Mosaic 2 in 1944 (three of these died in the same year) and one showed no symptoms, presumably because of a graft failure.



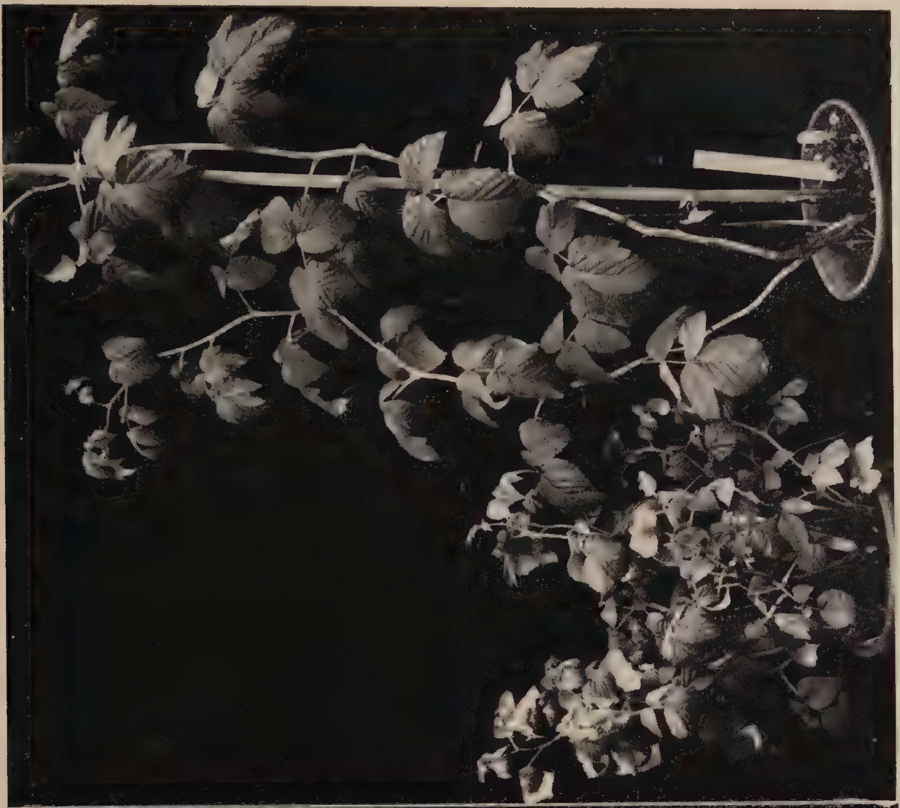


FIG. 1 and 2 Curly Dwarf on Baumforth's Seedling B from Lloyd George.

FIG. 1. One year after infection by grafting.
Left : normal. *Right* : infected.

FIG. 2. Two years after infection.
Right : normal. *Left* : infected.

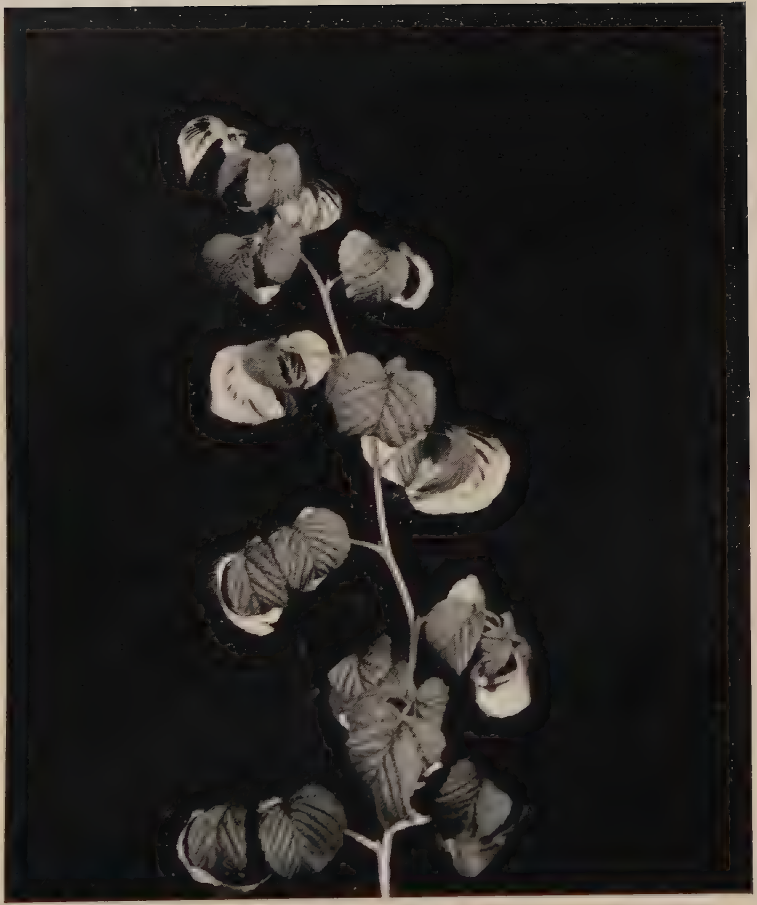


FIG. 3. Baumforth's Seedling B infected with Curly Dwarf from Newburgh.

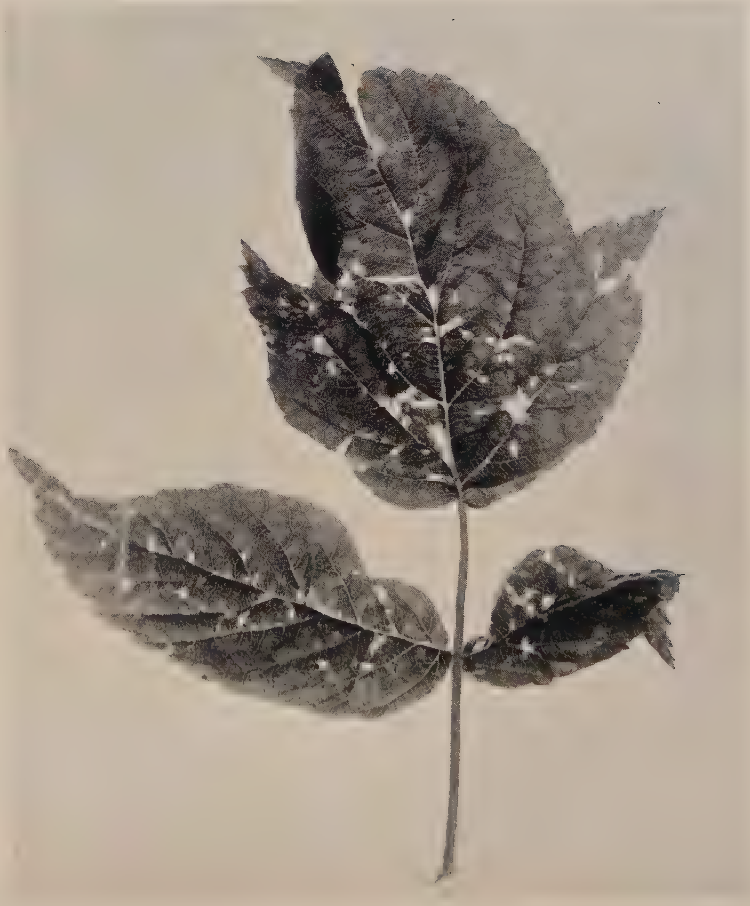


FIG. 4. Severe Mosaic 2 on Norfolk Giant.

These results confirm the previous work showing that Malling " B " is totally infected with Severe Mosaic 2, and show that this variety and Malling Landmark do not show diagnostic symptoms of this disease.

MALLING PROMISE.

Five plants of this variety were grafted to Baumforth B indicators in 1942 and five others in 1943. None of the indicators developed symptoms. Ten plants were grafted to Norfolk Giant indicators in 1946 and no symptoms were observed on the indicators. Ten plants were grafted to Baumforth B indicators in the same year and doubtful symptoms of Curly Dwarf developed on five of the indicators.

Ten plants of Malling Promise were grafted to Lloyd George infectors in 1946. The Lloyd George plants were from Selection A which earlier test (described above) had shown to be infected with Severe Mosaic 2. None of the Malling Promise plants developed diagnosable symptoms, but some appeared to be slightly reduced in vigour. It is therefore concluded that current stocks of Malling Promise were not at the time totally infected with virus and that this variety also is non-expressive of Severe Mosaic 2.

ANOMALOUS RESULTS WITH RED CROSS INFECTORS.

In 1942, seven Malling Landmark plants were grafted to infectors of the variety Red Cross. One graft failed, four of the Landmark plants developed a pronounced mosaic and were reduced in vigour and two showed no symptoms.

In 1943, three Malling Promise plants were grafted to Red Cross infectors. One Malling Promise plant developed mosaic symptoms and two remained symptomless. In 1944 these plants were regrafted to Baumforth B indicators. One of the Malling Promise plants continued to show mosaic symptoms and the others remained symptomless. All three of the Baumforth B indicators developed symptoms of Severe Mosaic 2, showing that the Malling Promise plants were all infected with Severe Mosaic 2.

As has been stated above, grafts of Malling Landmark and Malling Promise to other plants infected with Severe Mosaic 2 have shown that these varieties do not show symptoms when infected with this disease. It is thought, therefore, that the mosaic symptoms produced on some of the plants grafted to Red Cross were not due to Mosaic 2 but were caused by some other virus. It thus appears that the selection of Red Cross used, which had been previously shown to be uniformly infected with Mosaic 2 (Harris, 1940), was no longer uniformly infected and that a proportion of these Red Cross plants had become infected with some other additional virus since the original experiments of Harris.

DISCUSSION.

From the samples tested it appears that the majority of commercial stocks of Lloyd George that are free from symptoms of mosaic are, nevertheless, infected with one or more viruses. The infected state of such stocks gives support to the view that the reduction in vigour of this variety, which has been noted in recent years, is due to virus infection, including infection by viruses which do not produce symptoms of diagnostic value. The only satisfactory solution to the problem of deterioration in this variety would now appear to lie in the reintroduction of a virus-free selection.

The relations of the Curly Dwarf virus to other previously described viruses of *Rubus* are unknown. Curly Dwarf on Baumforth's Seedling B shows some resemblance to Blackberry Dwarf on Loganberry, etc., in that numerous weak canes are produced on infected plants. The viruses, however, are considered to be distinct for two Lloyd George plants infected with Curly Dwarf did not produce Blackberry Dwarf symptoms on plants of Phenomenal Berry to which they were inarched in 1946 nor did two loganberries with Blackberry Dwarf produce Curly Dwarf symptoms on Baumforth B.

The leaf-curling symptoms of Curly Dwarf suggest a resemblance to the Leaf Curl disease of raspberries common in America, but it is thought that the diseases are distinct. Harris (1935) reported that, in Canada, the Leaf Curl virus produced drastic symptoms on Lloyd George and Baumforth B and that infected plants died in a short time. The symptoms produced by Curly Dwarf in England are much less intense and the dark green, greasy appearance of the foliage that is characteristic of plants infected with Leaf Curl (Bennett, 1927) has not been observed. In 1946 five Lloyd George plants infected with Curly Dwarf were grafted to plants of Cumberland black raspberry, which is susceptible to the Beta Leaf Curl virus but not to the Alpha (Bennett, 1930). Three of the black raspberry plants were killed and the other two were stunted and very weak, but they did not develop any diagnosable symptoms.

On account of the differences of symptomatology, Curly Dwarf is also considered distinct from the Yellow Blotch-curl of Chamberlain (1938) and the Leaf Curl of Harris, Bryce and Foister (1943).

Cadman and Hill (1947) have reported the transmission to Norfolk Giant by *Doralis* (*Aphis*) *idaei* of a virus thought to be the Curly Dwarf virus.

SUMMARY.

(1) In the course of grafting tests of stocks of leading commercial varieties to *Baumforth's Seedling B* as indicator, a disease distinct from Mosaic 2 was identified in two of three cultures of Lloyd George derived from a single symptomless selection.

(2) The name " Curly Dwarf " is provisionally proposed for this disease which is considered, on available evidence, to be distinct from the American Leaf Curl disease, Yellow Blotch-curl in Canada, Leaf Curl in Scotland, and Blackberry Dwarf.

(3) From tests of a stock of *Norfolk Giant* it was concluded that this variety is a sensitive indicator of Mosaic 2.

(4) *Malling Seedling B* was confirmed to be totally infected with Mosaic 2, and this variety and *Malling Landmark* were found not to develop diagnostic symptoms of this disease.

(5) A stock of *Red Cross*, previously known to be uniformly infected with Mosaic 2, was found to have become partially infected with an additional and unidentified virus.

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THE CAUSE OF BLACK BLOTCH DISEASE OF THE RASPBERRY

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For many years it has been considered that Black Blotch disease of the raspberry was caused by the fungus *Cryptosporium minimum* Laub. which Harris (1931) had isolated from the superficial black spots or lesions that characterize the disease. He had been unable, however, to reproduce the symptoms on healthy canes by inoculation experiments and so it seemed likely that some other agent was primarily responsible for these symptoms. Evidence in support of this was obtained from 1942 onwards by Harris (unpublished) in the raspberry growing areas of south-eastern Scotland and in 1946, whilst examining, with the author, diseased Norfolk Giant canes in a garden at West Malling. It was noticed that canes affected with Black Blotch disease were supported by rusty iron wire, while those supported by new galvanized wire or not attached to wire were free from the black lesions. It was also observed that the black spots on the diseased canes were located on the cane below the point of attachment to the rusty wire, while few, if any, blotches occurred on the canes above this point. These observations indicated that a substance, possibly a metallic compound such as hydrated ferric oxide (iron rust) or zinc carbonate was being rain-washed from the rusty wire in sufficient amounts to cause the damage usually known as Black Blotch disease and that the fungus functioned merely as a saprophyte or a weak parasite.

It was therefore assumed that Black Blotch disease was a form of chemical injury and experiments were carried out to confirm this and identify the responsible metal. Later a trial was designed to find whether the symptoms of Black Blotch disease could be induced on canes by the application of pure salts of the suspected metal.

EXPERIMENTAL RESULTS.

Identification of metals associated with the disease.

Samples of rusty wire taken from a row of raspberries suffering from the disease were subjected to chemical analysis ; iron was the only element found. Samples of un-rusted galvanized wire which had supported healthy canes in the same garden were also examined ; zinc and iron were present, together with traces of lead.

The bark from diseased and healthy Norfolk Giant canes supported by rusty and galvanized wire respectively, was then subjected to chemical and spectrographic analysis. Three canes from the same garden were examined. The first (cane A) had been tied to rusty iron wire and showed typical symptoms of Black Blotch disease, the second (cane B) had been trained on un-rusted galvanized wire in another part of the garden and appeared to be free from the disease. In both cases, bark was removed for analysis from an area below the point of attachment to the wire. The third cane (cane C) had also been tied to rusty wire, but the spots were much less numerous above the point of attachment than below it. Bark was removed from this cane, above and below the level of the wire and subjected to analysis.

TABLE I.

Chemical analysis of tissue taken from healthy and diseased Norfolk Giant raspberry canes.

Source of tissue.	Metals determined as sulphides, expressed as per cent. of dry bark.
Cane A, blotched area below rusty wire	4·25
Cane B, healthy area below galvanized wire	0·27
Cane C, blotched area below rusty wire	3·70
Cane C, healthy area above rusty wire	1·30

It will be seen from these results that the metallic content of the bark from the diseased cane A was much higher than that from the apparently healthy cane B and that the diseased part of cane C was richer in metals than the healthy part.

Spectrographic analysis (see Fig. I) of tissue taken from the three canes showed that bark taken from below the rusty wire was considerably richer in iron than bark taken from below un-rusted galvanized wire. A comparison was also made of the spectrograms of tissue taken from the same cane above and below the point of contact with rusty iron wire ; a higher iron content was again associated with the presence of diseased blotches. These results provided strong evidence that compounds of iron cause chemical injuries to the cane which result in the formation of black spots and blotches. An attempt was then made to induce the symptoms artificially on normal canes both by the attachment of rusty wire and by the application of iron salts.

Identification of metal causing the disease.

Sixty Norfolk Giant raspberry plants were raised in pots for the trial and were tied by raffia to bamboo stakes for support. The treatments, which were applied to the fruiting canes, included the application of suspensions of finely divided metals and solutions of metallic salts in distilled water, to all parts of the plant by means of an "Eclipse" hand sprayer, care being taken that drift from one treatment did not fall on canes under another treatment. The rusty and galvanized wire was fixed in place by looping a 5-inch length of the wire around the cane 18 inches above ground level. In addition to iron, zinc was included in the treatments as this metal might be suspected of causing the disease. The twelve treatments were applied on 25th April, 1948, each to five potted plants. The weather was fine and dry during the spray application ; no rainfall occurred until 28th April when 0·14 in. was recorded. Thereafter rain fell each day (average 0·07 in. per day) until 3rd May, when symptoms of the disease had appeared on all the plants receiving treatments which included iron. Observations on the effect of the treatments were continued for four months, and are given in Table II. (See Figs. 2-5.) As the results were clear cut and the replicates agreed closely, it was not considered necessary to record the results numerically.

The trial showed that the canes and laterals of Norfolk Giant raspberry were very susceptible to chemical injury by the element iron, and that the form of the injury observed was indistinguishable from that known as Black Blotch disease. It was

TABLE II.

Results of trial on Norfolk Giant raspberry to determine which metal caused symptoms of Black Blotch disease when applied to healthy fruiting canes.

Treatment applied 25/4/48.	Visible effects of treatment and date of observation.			
	28/4/48.	3/5/48.	8/6/48.	24/8/48.
0·1 per cent. iron powder (pure)	None	Minute black spots on cane only	As 3/5/48	Many small spots on cane
0·1 per cent. iron and zinc powder	None	Minute black spots on cane only	As 3/5/48	Many small spots on cane
0·1 per cent. zinc powder (pure)	None	None	None	No blotch
2·5 per cent. zinc sulphate (A.R. $ZnSO_4.7H_2O$)	None	No spots, some leaf scorch	No spots, slight defoliation	No blotch. Some defoliation
0·25 per cent. zinc sulphate	None	None	None	No blotch
0·025 per cent. zinc sulphate	None	None	None	No blotch
5·0 per cent. ferric sulphate (pure $Fe_2(SO_4)_3.9H_2O$)	Black blotches on cane and laterals. Severe leaf scorch.	Black blotches on cane and laterals. Some laterals surviving treatment.	Complete symptoms of Black Blotch disease. Plants weakened but surviving.	Severe Black Blotch. Fungus absent.
0·5 per cent. ferric sulphate (pure $Fe_2(SO_4)_3.9H_2O$)	Black spots on cane. Slight leaf scorch.	Black spots on cane. Few black spots on foliage.	Black spots on cane.	Symptoms of Black Blotch present.
0·05 per cent. ferric sulphate (pure $Fe_2(SO_4)_3.9H_2O$)	None	Many small black spots on cane only. No scorch.	Many small black spots on cane only.	Mild symptoms of Black Blotch.
Rusty wire	None	Black spots on cane for 2 in. below rusty wire.	Black spots on cane below rusty wire.	Typical Black Blotch symptoms.
Galvanized wire	None	None	None	None
Control	None	None	None	None

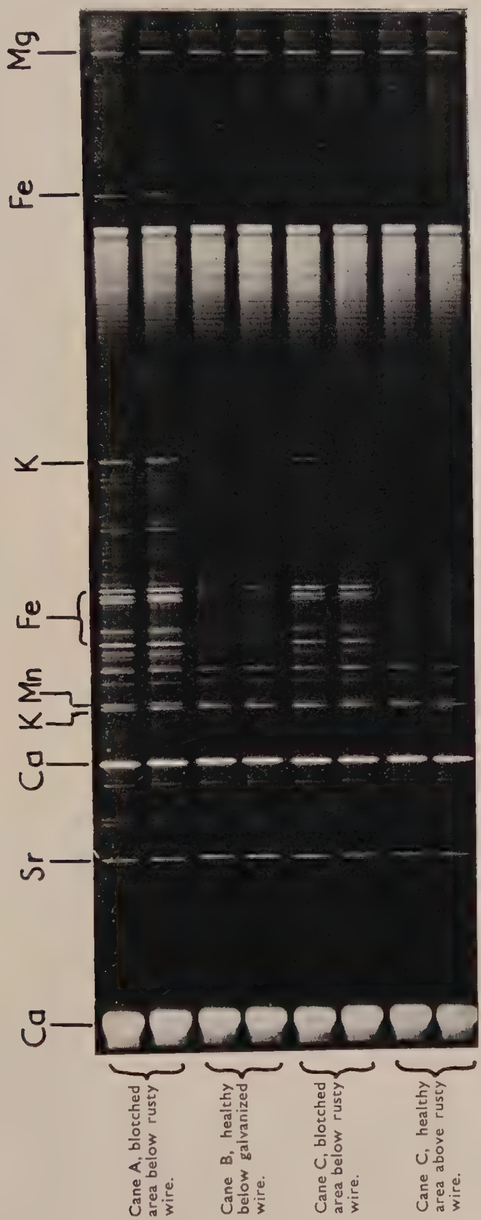

FIG. 1. Spectrogram of tissue taken from Norfolk Giant raspberry canes, showing high iron content of tissue from blotched areas of the plant.

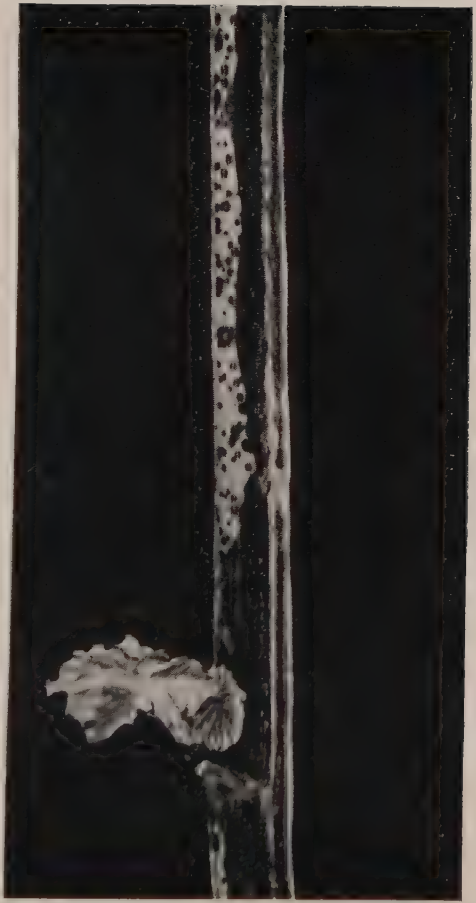

FIG. 2.
Black Blotch disease naturally occurring on Norfolk Giant cane.

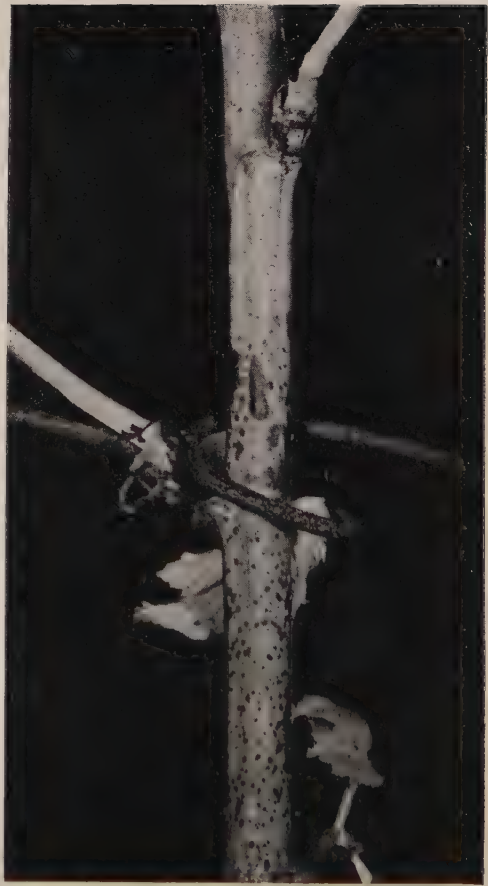

FIG. 3.
Black Blotch disease artificially induced on Norfolk Giant cane. Photographed six weeks after attachment of rusty iron wire.

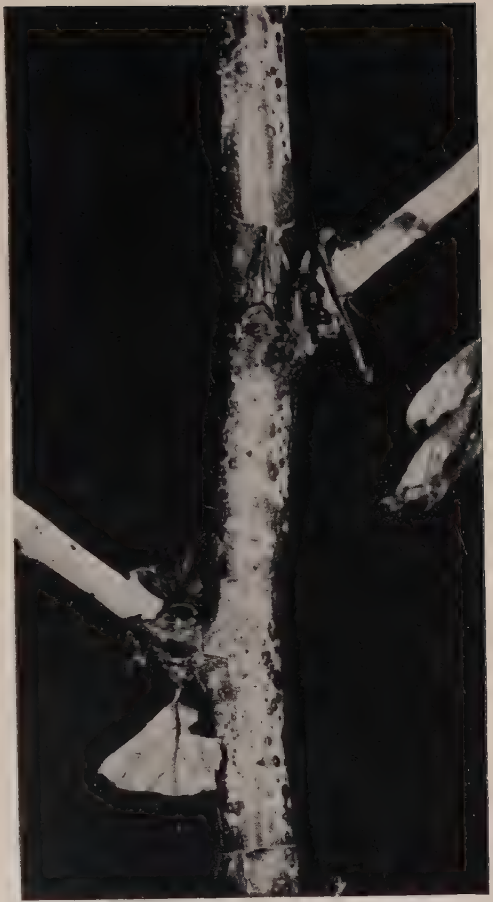

Fig. 4.
Black Blotch disease artificially induced on Norfolk Giant cane. Photographed six weeks after spraying with 5 per cent. ferric sulphate solution.

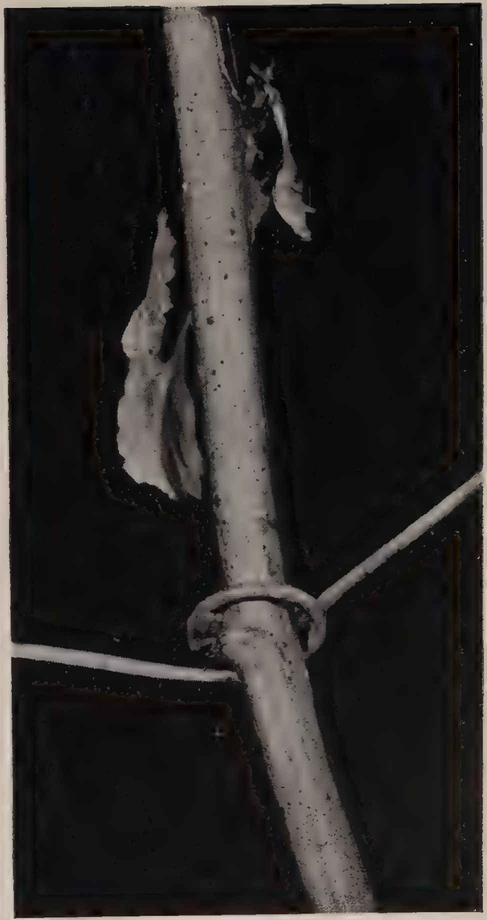

Fig. 5.
Healthy appearance of Norfolk Giant cane. Photographed six weeks after attachment of new galvanised iron wire.

also shown that no symptoms of the disease were caused by zinc whether in the form of zinc dust, zinc sulphate or even zinc coated (galvanized) iron wire.

Examination of black blotches for presence of fungi.

The superficial black blotches produced by spraying with ferric sulphate were examined on two occasions for the presence of fungi, but none was found.

DISCUSSION.

It is well known that plants of different species vary widely in their tolerance to metals. Thus Moore (1938) showed that Bordeaux mixture at a strength which was safe for use on hops caused severe damage when applied to the foliage and fruit of apple. No critical information is available on the concentration of iron necessary to cause damage to various plants, but it would appear from the data presented above that the raspberry is sensitive to iron. A disease of cucumber plants has been recorded by Caldwell (1935) as being caused by traces of copper ; the symptoms were mottling of the leaves and it appeared that the copper had interfered with chlorophyll formation. In the present case the damage appears to be localized injury of the outer tissue of the cane, caused by rain splashes charged with the iron compound. It has been shown that the characteristic black blotches are produced in the absence of the fungus, but as infection by *Cryptosporium minimum* Laub. did not occur during the experiment, it was not possible to determine whether or not the toxic effect of these iron compounds is intensified by subsequent fungal action. In some field cases examined, incidence of the blotching was associated with a subnormal development of fruiting laterals and even with death of a part of the canes but so far no experimental verification of this is available. It should be emphasized in this connection that the observed absence of inhibitory effect on lateral development in the experiment may be related to the fact that treatments were applied (25th April) some 2 to 3 months after the fruit canes would normally have been tied to their supporting wires.

SUMMARY.

During an investigation of the Black Blotch disease of raspberries formerly attributed to the fungus *Cryptosporium minimum* Laub., it was found that the lesions are primarily caused by the toxic action of iron compounds distributed by rain from rusty iron wire upon which the affected canes are trained.

ACKNOWLEDGMENTS.

The author is grateful to Dr. R. V. Harris for his advice and interest during the investigation and to Dr. W. A. Roach for permission to include analytical data obtained by the Biochemistry Section.

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A DUAL TEMPERATURE METHOD FOR THE REFRIGERATED CARRIAGE OF PLUMS

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Plums form a not insignificant proportion of the total export of deciduous fruits from the Union of South Africa and they have for long presented a special difficulty in overseas transportation. The difficulty is not simply one of perishability. By picking the plums at a suitable maturity, pre-cooling them immediately and rapidly to about 31° F., and carrying them overseas at this temperature, it is possible to avoid serious wastage of the more obvious kind. But much of the fruit treated in this way will not subsequently complete its ripening in a normal fashion at English winter temperatures. When first received it may be quite suitable for culinary purposes. Further storage in the merchant's warehouse, instead of producing a good dessert fruit, results eventually in the appearance of one or other of various types of tissue-breakdown which impair even the culinary quality. The problem presented was to devise a shipping treatment combining reasonable insurance against damage by over-ripeness and mould with reasonable preservation of the capacity to ripen subsequently under English winter conditions.

The two principal varieties exported from South Africa are the Santa Rosa which is shipped in December and early January, and the Kelsey whose season begins in mid-January and lasts until the end of March. During the five-year period from 1934/35 to 1938/39, inclusive, Santa Rosas constituted just under 28 per cent. and Kelseys about 34 per cent. of the total cubic tonnage of plums exported. The other varieties, in order of importance, were Gaviota (16·6 per cent.), Wickson (5·5 per cent.), and Prune (4·8 per cent.), followed by Satsuma, Methley, Apple, Beauty and several others.

Course of Ripening.

The visible course of the ripening process in the two main varieties is very briefly described below.

Santa Rosa :

Maturity in Santa Rosa plums can be accurately judged by the colour of the skin, which changes fairly rapidly from green to dark red, through stages which it is often convenient to differentiate as follows : (i) green and up to $\frac{1}{4}$ red, (ii) $\frac{1}{4}$ to $\frac{3}{4}$ red, (iii) $\frac{3}{4}$ to full light red, (iv) mid- to dark red. Fruits picked at all stages of maturity, other than the very earliest, will ripen normally when they are held at atmospheric temperatures in Cape Town, which average about 75° F. at this time of the year. Under these conditions the fruit passes from any one stage to the next in about $1\frac{1}{2}$ days, and it usually remains in sound, ripe condition for five or six days after reaching the final dark red (maroon) colour. The typical maturity at picking for export is the stage at which the red flush on the skin covers about three-quarters of the surface area ; but two or three, occasionally all four, of the above stages may be found in a single consignment when care has not been taken to separate them. During pre-cooling and transport at 31° F. the colour advances by about half a stage in the period of 2½ to 3 weeks. After storage at 31° F. for this time, most of the fruits fail to complete their ripening at the temperature of 45° F. which is, broadly speaking, typical for fruit warehouses in the United Kingdom at this time of the year ; or if not they ripen so slowly that physiological breakdown intervenes. These plums could often be ripened normally if the warehouse temperature were raised to 65° F. or above until ripening was well begun. Fruits shipped at stage (iv), particularly if they have been picked from trees which have recently been watered by rain or irrigation, are prone to the form of breakdown which is expressively termed " bladdery ".

Kelsey :

Under normal circumstances the Kelsey plum ripens slowly, or not at all. It is usually picked for export at a stage when the ground-colour of the skin is yellowish green, with perhaps a trace of red at the apex of the fruit or along the suture. At this stage the flesh is relatively tough and lacking in juice. In many cases it is probable that maturation would not advance appreciably beyond this stage, no matter how long the fruit was left hanging on the tree. It also usually fails to ripen when picked and held at atmospheric temperatures in Cape Town. An apparently normal sample of fruits, initially uniform in appearance, will contain a few which subsequently ripen and a great majority which do not. Sometimes one part of the plum will colour up, soften and become juicy—often apparently as a consequence of " Kelsey spot ", insect puncture or other abnormality—while the rest does not. Ripening capacity bears some relation to the total content of dissolved substances (T.D.S.), but not to the acid content, which is low at all stages. The T.D.S. content at picking can vary from as little as 10 to as much as 20 per cent., but typically perhaps is about 12 per cent. Fruits left on the tree for some time after full growth has been attained, in the hope of increasing the capacity to ripen, are often subject to serious losses from wind, sun-burn, pitting and insect punctures.

The Kelsey plums carried overseas at 31° F. or slightly higher temperatures, and then stored at warehouse temperatures (about 50° F. at this time of the season) in England, will probably ripen a little more uniformly and more effectively than would the same fruit if held throughout at South African atmospheric temperatures. Tests made in 1937/38 (5) indicated that on the average about one plum in ten from commercial export consignments ripened when held in South Africa (at 65°-75° F.), whereas about one plum in four from the same consignments ripened after transport to England. These figures are sufficient in themselves to emphasize the magnitude of the difficulty which has to be faced.

Other varieties :

The Gaviota plum resembles the Kelsey in that it frequently fails to ripen when picked at normal maturity for export and held at South African temperatures, but it ripens relatively well in the United Kingdom after carriage overseas at 31° F. or slightly above. The Wickson plum is rarely seen as a fully ripe fruit, either in South Africa or in the export trade. The pointed apex of this fruit sometimes ripens well ahead of the rest, and may become excessively soft at a time when the greater part of the plum is barely soft enough or juicy enough to be edible.

CARRIAGE AT HIGHER TEMPERATURES.

The possibility of carrying these plums overseas more successfully at some higher temperature was first investigated by Rees Davies and colleagues (1) at the Low Temperature Research Laboratory, Cape Town. Their results showed that no improvement was to be anticipated in a range of storage temperatures between 31° F. at one extreme and 40° to 45° F. at the other extreme. It was demonstrated, however, that under laboratory conditions Santa Rosa plums could often be preserved at 45° F. for a period of about five weeks, which would permit of transport overseas and marketing in England, and that they would in the interval ripen in a normal fashion, without serious internal breakdown and with fair ultimate flavour. It was shown also that Kelsey plums could often be preserved for a similar length of time at 48° F., and would progress to edible maturity without internal breakdown and with fair ultimate colour and flavour, if they had been picked initially at a suitable maturity-stage.

Experimental shipments at these temperatures were made in 1935/36 and succeeding years (10). The first shipment of Kelsey plums at 45° F. gave a material improvement as regards arrival quality, but it was noted that the colour and eating-quality did not continue to improve after discharge from the ship ; instead, the plums slowly lost flavour and juiciness and in the end developed internal injury. In a larger shipment made at this temperature, later in the season, much of the fruit was excessively soft when it arrived. In the following season seven large shipments of Kelsey plums were made at 45° F. Five of them were reported as " variable but on the whole moderately successful ", but two showed heavy wastage from the presence of abnormally soft fruits and from mould attack. In 1937/38 three small shipments were made at the slightly higher temperature of 48° F. Only about 20 per cent. of the fruits examined from these three shipments were classified as firm enough on arrival to permit safe commercial handling, and in one shipment 50 per cent. of the fruit was so soft as to be virtually unsaleable.

The first shipment of Santa Rosa plums at 45° F. (in 1936/37) was moderately successful, but the fruit was judged to be generally in too soft a condition for satisfactory marketing, and there was considerable mould in some boxes. A repetition of the trial in the following season produced similar complaints.

RIPENING BY ACETYLENE.

One possible explanation of the contradiction which appeared between laboratory results on a small scale and commercial experience on a larger scale, was afforded later by the observation of Putterill and Dreyer (5) that unripe Kelsey plums could be ripened by enclosure for a time with naturally ripened fruits in the same container. It follows that the average rate of ripening may be greater when fruits are massed together, in a chamber with restricted ventilation, than it is when small, uniform samples are stored in the laboratory. Isaac (3) had shown that a metabolically active substance, probably ethylene, is produced by ripening plums, as is the case with apples and bananas and several other fruits. It had also been shown by Putterill (4), and was confirmed shortly after by Davies and Boyes (2), that treatment with small concentrations of acetylene had a remarkable stimulating effect on the ripening of Kelsey plums, and some other varieties (5). This method of treatment was adopted with little delay, and on some considerable scale, in the treatment of plums for marketing inside the Union, and was also experimented with as an export possibility.

The difficulty here was twofold: if the plums were treated before export and then carried at 31° F. the flesh tended to break down and become bladdery, and the normal change in colour of the skin from green or greenish-yellow to deep red was largely inhibited; while if the treatment were delayed until the plums arrived in the United Kingdom, after carriage at 31° F., it could only be carried out with success at artificially high temperatures, and even so the reaction was comparatively sluggish. It should, nevertheless, be borne in mind that carriage at 31° F., followed by artificial ripening at a high temperature in the presence of ethylene or acetylene, may be technically the most attractive of all possibilities for the satisfactory ripening of the Kelsey plum.

THE DUAL TEMPERATURE METHOD.

An alternative possibility which appeared to merit investigation was that of changing the temperature in the ship's refrigerated chamber during the period of carriage. The theoretical basis for this treatment derived from the assumption that while capacity to ripen is indubitably injured sooner or later by low temperatures, the injury is not immediate, or at least is not in its earliest stages irreversible. This is a reasonable deduction from what has been said above, namely that plums stored for three weeks at 31° F. can be ripened subsequently at 65° to 75° F., either in air or (more readily) with the help of acetylene or ethylene. Fair ripening could be obtained by storage continuously at 45° to 50° F., but in commercial practice these plums arrived too soft, and the risk of extensive mould damage was too great. Temperatures intermediate between 31° F. and 45° F. had shown no advantage, and merely tended to produce a more rapid visible development of the low temperature injury. What remained for investigation was whether storage at 31° F. for a shortened period would, or would not, so damage the physiological mechanism as to prevent ripening at 45° F. or 50° F. subsequently.

Laboratory experiments to test this possibility were made with Kelsey plums by Dr. I. Donen of the University of Cape Town, in collaboration with the author, during the season 1936/37. At this stage it was not clear whether the better result would be obtained from the use of a low temperature followed by a higher one, or by the reversed order of procedure, which is adopted in what are often called "delayed cold-storage" treatments. The laboratory results showed particular promise in the use of a low temperature followed by a higher one.

Trial shipments were commenced in the following year (1937/38), and they were pursued very actively during the 1938/39 season, when about a quarter of the whole export of Santa Rosa and Kelsey plums was carried overseas by the new method. At this stage the results of Tindale, Huelin and Trout (9) with Australian plums became available, and it was clear that these workers had arrived independently at similar conclusions, although the time-scale of their suggested procedure was very different from that which offered a possibility of success with South African plums.

SMALL-SCALE TESTS WITH KELSEY PLUMS.

Small-scale laboratory tests were also made (7), on behalf of the Perishable Products Export Control Board, in 1937/38 and 1938/39. They were conducted in experimental rooms attached to the Pre-cooling Store at Table Bay harbour, and were devoted primarily to investigating the effects of various times of storage at 31° F. on the ability of the fruit to ripen, and its final condition, at 45° or 50° F. Table I, for example, shows the effect of various dual temperature treatments (31°/50° F.) on the eventual firmness of Kelsey plums in the 1938/39 season. The plums marked " very soft " were obviously unfit for commercial marketing, and those marked " rather soft " were borderline in this respect. (The other categories of classification were " firm ", and " fairly firm ".) The numbers given are average percentages for 15 different consignments, which were chosen at intervals during the season, and from the various growing districts. Each consignment was divided into four experimental lots for the different temperature treatments. This work was carried out jointly by the author and by Mr. D. C. Ferguson of the technical staff of the South African Co-operative Deciduous Fruit Exchange.

TABLE I.

Dual temperature treatments in relation to softening in Kelsey plums (1938/39).

Treatment.	Percentage : Rather soft.	Percentage : Very soft.	Total.
Total storage, 4 weeks :			
50° F. throughout	14	8	22
5 days at 31°, then 50° F. ..	12	3	15
10 days at 31°, then 50° F. ..	0	2	2
15 days at 31°, then 50° F. ..	0	1	1
21 days at 31°, then 50° F. ..	0	0	0
Total storage, 5 weeks :			
50° F. throughout	17	46	63
5 days at 31°, then 50° F. ..	22	22	44
10 days at 31°, then 50° F. ..	17	3	20
21 days at 31°, then 50° F. ..	4	1	5

The individual samples differed rather widely in the retention of their ripening capacity under a given set of storage conditions. Some samples, which apparently had been picked at a rather immature stage, did not ripen satisfactorily even when held at 50° F. for the whole five weeks; but one or two samples were so broken down as to be almost unsaleable after four weeks at this temperature. Speaking generally the samples which were kept for five days at 31° F. ripened satisfactorily, and not too rapidly, at 50° F. After ten days at 31° F. a small proportion of the plums in a sample usually failed to complete their ripening, but the loss on this account was never serious in fruits which appeared to have been picked at relatively advanced maturity. After 15 days at 31° F. a notable proportion of the fruits failed to ripen, and often developed internal injury, during the following period at 50° F. After three weeks at 31° F. few fruits, even from maturely-picked consignments, ever reached a soft, juicy texture at 50° F.

TEST SHIPMENTS.

The trial shipments in 1937/38, and again on a very much larger scale in 1938/39, were also made the occasion of experimental study. The small test samples which accompanied these shipments were selected and examined with great care by the staff of the Chief Fruit Inspector at Cape Town. Each test sample consisted of two similar lots of fruit, usually in double-layered trays containing from 80 to 150 plums, according to the size-grading. The fruit was selected from a commercial consignment when it arrived at the pre-cooling store, usually within 24 hours of the time of picking. The plums from two similar boxes, already of a quality and uniformity prescribed by export regulations, were unpacked and mixed, and were then re-sorted into classes of maturity as judged by appearance and colour. After this they were divided again to make two strictly comparable samples, with equal numbers of each maturity in each sample, and these were packed back into the two original boxes, with a clear demarcation of the different maturity-classes for help in examination overseas. After pre-cooling to a temperature as near 31° F. as possible (using air at 30° F. moving at high speed in a tunnel) the two boxes of fruit were shipped overseas in the same vessel, one in a chamber at 31° F. and the other in a chamber whose temperature was to be raised to 45° or 50° F. as from a prescribed date. The selection and re-sorting of the fruit was superintended by Mr. D. J. Dreyer of the Chief Fruit Inspector's staff.

The experimental boxes were consigned to the Overseas Representative of the Deciduous Fruit Exchange, and on arrival in England were examined by the Technical Officer of the Exchange, Mr. P. G. H. Barter, and his colleagues. They were then held at atmospheric temperatures in London, and were re-examined periodically to assess the progress of ripening. At the final examination all the fruits were cut open in order to enumerate and classify internal abnormalities.

The consignments were chosen at various times during the plum season, and were selected to be as representative as possible of the various growing districts. The total number of paired consignments sent overseas in 1938/39 exceeded 120. The data obtained during this season will be summarized below.

Santa Rosa Plums.

Sixty-two paired samples were sent overseas, representing a total of nearly 20,000 fruits, from eight different growing districts. The percentages of fruit in

TABLE II.

Santa Rosa plums carried at 31° F. and at 31°/45° F. (1938/39).

	31° F.	31°/45° F.
Wastage on Arrival:		
Bladdery	803	543
Mould	18	38
Ripening of the Sound Fruit:		
Ripe within 1 week.	1,061	4,730
Ripening in 2 to 3 weeks	3,543	3,653
Unripe after 3 weeks	4,253	474
Final Condition (after 3 weeks):		
Additional mould	16	6
Internal breakdown	2,152	264
Unripe, sound	2,628	410
Ripe, sound	3,240	7,596

different colour-stages at the time of arrival at the pre-cooling store were as follows: trace to half red 5·8 per cent., half to nearly full red 50·5 per cent., full light red 36·2 per cent., dark red 7·2 per cent. All samples were held in the pre-cooling store at approximately 31° F., but the "dual temperature" boxes were allowed to warm to 45° F. in the ship's chamber at the outset of the voyage. The main results are set out in Table II.

Most of the results were fairly uniform from one growing district to another, but it is noteworthy that a high proportion of the bladdery plums (at both temperatures) was found in samples from the Groot Drakenstein district and (to a smaller extent) the Fransch Hoek area. The plums which arrived bladdery were nearly always those which had been at the dark red stage when first received at the pre-cooling store. In Table III the data have been classified according to the initial maturity, A representing trace to half red, B half- to not quite full red, C full light red, and D mid- to dark red.

TABLE III.

Santa Rosa plums (1938/39) classified by maturity.

Maturity	A	B	C	D
Total number of fruits	462	4,055	2,914	571
After carriage at 31° F.:				
% Bladdery	3	1	13	81
% Mould	—	1	—	—
% Internal breakdown	40	24	23	4
% Ripening normally, within 3 weeks ..	6	34	48	12
% Sound, but unripe after 3 weeks ..	51	40	16	3
After carriage at 31°/50° F.:				
% Bladdery	1	1	8	64
% Mould	1	—	1	—
% Internal breakdown	2	2	4	5
% Ripening normally within 3 weeks ..	79	89	86	31
% Sound but unripe after 3 weeks ..	17	8	1	—

TABLE IV.

Gaviota plums carried at 31° F. and at 31°/45° F. (1938/39).

	31° F.	31°/45° F.
Wastage on Arrival:		
Bladdery	10	16
Mould	3	1
Ripening of the Sound Fruits:		
Ripe within 1 week..	20	407
Ripening in 2 to 3 weeks	920	951
Unripe after 3 weeks	439	25
Final Condition (after 3 weeks):		
Additional mould	2	1
Internal breakdown	249	91
Unripe, sound	224	7
Ripe, sound	893	1,264

Gaviota plums.

Eleven paired samples were examined, representing in all about 2,800 fruits. Two consignments initially contained a high proportion of plums at the yellowish-red stage, and one consignment contained a small proportion of wholly green fruits. The rest were classified initially as belonging to one or other of two intermediate stages, viz. yellowish green with pink colour at the apex (56 per cent.), or greenish yellow flushed with red (27 per cent.). The data are summarized in Table IV.

Consignments from different farms differed greatly in their ability to ripen (within 3 weeks) after carriage at 31° F. for the whole voyage. If attention is confined to the five samples in which there was a serious failure to ripen, the individual results were as summarized in Table V.

TABLE V.

Gaviota plums which ripened with difficulty after carriage at 31° F. throughout (1938/39).

District *	Cer.	Bhk.	Fhk.	Cer.	Bhk.
Fruits per Tray	168	96	123	168	132
After carriage at 31° F.:					
Ripe within 1 week..	0	3	0	0	0
Ripening in 2 to 3 weeks	137	57	53	25	5
Unripe after 3 weeks	31	36	70	143	127
Internal breakdown after 3 weeks	39	44	28	7	90
After carriage at 31°/45° F.:					
Ripe within 1 week..	9	96	37	15	0
Ripening in 2 to 3 weeks	156	0	86	144	130
Unripe after 3 weeks	3	0	0	9	2
Internal breakdown after 3 weeks ..	7	9	11	6	33

* Cer. — Ceres; Bhk. — Banhoek; Fhk. — Fransch Hoek.

Wickson plums.

Thirteen paired samples, or about 3,200 plums in all, were examined. The initial maturity stage was that of a green to yellowish green ground colour; a few plums in some consignments (6 per cent. altogether) showed traces of red colour at the apex or along the suture. The dual temperature treatment was the same as that used for Santa Rosa and Gaviota plums, viz. 31° F. in the pre-cooling store and 45° F. on the ship. In three of the six consignments from Ceres an appreciable proportion of the plums reached edible ripeness, without internal breakdown, within three weeks of arrival in England after carriage at 31° F.; the results for these three consignments (under the heading Ceres A) are shown separately from those for the other three (Ceres B) in Table VI.

Kelsey plums.

Thirty-five paired samples of Kelsey plums, representing a total of about 5,300 fruits, were selected at various times between the end of January and the beginning of March, from export consignments from the following districts: Fransch Hoek (11), Groot Drakenstein (5), Stellenbosch (5), Banhoek (2), Wellington (1), Ceres (6), Elgin (4), Piquetberg (1). Judged by colour the maturity was variable. Two samples contained less than 20 per cent. of fruits in colour-stage (i) (green to yellowish green),

TABLE VI.

Wickson plums carried at 31° F. and at 31°/45° F. (1938/39).

District	Paarl.	Wols.	Ceres. A	Ceres. B
Consignments	3	4	3	3
After carriage at 31° F.:				
Soft breakdown on arrival	47	105	28	23
Mould on arrival or later	8	0	0	0
Condition after 3 weeks:				
Leathery	104	44	2	35
Internal breakdown	209	293	211	216
Unripe, sound	2	4	6	42
Ripe, sound	13	49	156	31
After carriage at 31°/45° F.:				
Soft breakdown on arrival	6	8	6	2
Mould on arrival or later	1	0	0	0
Condition after 3 weeks:				
Leathery	0	0	0	0
Internal breakdown	27	60	21	60
Unripe, sound	58	27	11	57
Ripe, sound	291	403	365	228

TABLE VII.

Kelsey plums carried at 31° F. and at 31°/50° F. (1938/39).

District	Fr. Hk.	Gr. Dr.	Stell.*	Ceres.	Elgin.
Consignments	11	5	7	6	4
Total number of fruits	2,122	740	914	686	516
Av. % initially at stage 1	69	48	81	73	55
After carriage at 31° F.:					
Very soft or bladdery	17	10	2	11	12
Leathery	82	8	40	17	10
Internal breakdown	481	127	207	210	178
Unripe, sound	298	59	82	0	25
Ripe, sound	179	141	119	88	17
After carriage at 31°/50° F.:					
Very soft or bladdery	118	57	18	55	20
Leathery	0	0	0	0	1
Internal breakdown	149	59	58	101	82
Unripe, sound	156	8	90	0	55
Ripe, sound	610	243	290	180	95
% ripening normally:					
After 31° F.	17	38	26	27	7
After 31°/50° F.	59	66	63	53	36

* Stellenbosch, incl. Banhoek (2).

but eight consignments consisted solely of fruits at this stage. It is clear, however, that the presence of red colour is not a reliable index of maturity in this variety. All the fruit was pre-cooled to as near 31° F. as possible, and the dual temperature fruit was carried at 31° F. on board ship for two, three or four days from the time of sailing. The aim was to keep the total low temperature period within a limit of ten or eleven days. About 48 hours was required to raise the temperature to 50° F., and thereafter it was maintained at this level until discharge at Southampton. All the samples were held for five weeks at atmospheric temperatures in England. They were examined at intervals, but only final results are shown, above, in Table VII.

These data for Kelsey plums can be summarized, perhaps more expressively, in the following way, which shows the number of consignments in which a given proportion of the fruits eventually ripened :

	At 31° F.	*31°/50° F.*
Percentage eventually ripening :		
Less than 10	10	nil
Between 10 and 30	12	1
Between 30 and 50	9	11
Between 50 and 70	2	14
More than 70	2	9

Taking average figures for the whole of the 35 consignments, 24 per cent. of the fruits ripened without injury after carriage at 31° F., and 58 per cent. after carriage at 31°/50° F. The variation in final condition between individual fruits in a box was also considerably reduced by the dual temperature treatment.

DISCUSSION.

Regarded as a whole, these results were encouraging. With the Kelsey plum small-scale experiments had already made it clear that the particular dual temperature treatment which it was thought safe to adopt was not likely to provide more than a partial solution. The possibility had always to be borne in mind that at the higher carrying temperatures, in a ship's space which is stowed tightly and ventilated only with discrimination, the production of ethylene by a small proportion of ripening fruits might become a significant factor in governing the general condition of the outturn.

There were other circumstances which dictated some circumspection in adopting the dual temperature method as a standard practice. In the first place the small experimental consignments necessarily consisted of sound, unblemished fruit which had been carefully selected and expertly packed, and it could not be assumed that all the export fruit would be as free from damage by mould and fungal rotting at the higher carrying temperatures as the test consignments had been. The shipments previously made with higher carrying temperatures throughout the voyage, had clearly shown that better ripening capacity might be dearly purchased at the cost of increased fungal wastage. An unripe plum can be cooked, but a rotted plum is of no value whatever.

Moreover, there are great seasonal differences in the ability of some varieties of plums to ripen, or in the rate of ripening. In particular the Santa Rosa plum colours and softens with extreme rapidity if an excess of water is supplied to the tree in the final period of maturation, and plums ripening under these conditions are especially liable to become bladdery in storage. The presence of a small proportion of bladdery plums may greatly increase the risk of mould damage in the rest of the box, and also in neighbouring boxes of fruit.

Again it is impossible to segregate on the carrying vessel each variety of plum, or each consignment which might profit from a special treatment. The smallest independently controlled refrigerated space is likely to have a capacity of about 100 cubic tons, and even if reserved specifically for plums it will usually have to carry a mixture of varieties, all of which must clearly be subjected to the same temperature treatment while they are on board.

Since pickings are made daily, and shipments as a rule only weekly, even the plums of a single variety in a single chamber will not have identically the same low temperature history. For example the plums which are picked on Wednesday, and received at the pre-cooling store on Thursday, can sometimes be cooled for shipment in the mail vessel which leaves on Friday ; but frequently they may have to wait for the vessel which leaves a week later. Part of the cargo in any one refrigerated space may have been cold for only a few hours, and another part may already have been cold for more than a week, at the time when the vessel sails. But if the temperature is to be raised during the voyage it will be raised at the same time for all the fruit. In each individual case the risk of holding some plums at the low temperature for too long a time must be balanced against the risk of holding others, in the same chamber, for too short a time.

It was originally decided that during the 1939/40 season all Santa Rosa plums, and all wrapped Kelsey and Wickson plums, would be carried by a dual temperature method. The wrapped plums were assumed to be the least likely to be affected by the spread of mould, and to be the fruits in which full ultimate dessert quality was especially desired. With the outbreak of war a new factor entered, and the shortage of sugar supplies in the United Kingdom led to anticipations that culinary fruit would be saleable only with difficulty. In consequence it was decided also to use the dual temperature treatment for unwrapped Kelsey and Wickson plums, and to continue experimentation with several other varieties.

The general procedure in 1939/40 followed closely upon the lines adopted for Kelsey plums in the trial shipments of 1938/39. After being shipped at as low a temperature as possible (usually between 30° and 33° F.) the dual temperature fruit was carried at 31° F. for another four, five or six days, according to circumstances. At the specified time cooling was discontinued and the space was warmed to the higher temperature (45° or 50° F.) with the aid of heat from the fans, which were run at a fast speed. The new temperature was reached in 48 hours or less, and was then maintained for the rest of the voyage.

The results were again gratifying. Losses from overripe plums, bladdery plums and mould were relatively small. Ripening after arrival was a little less satisfactory than in the experimental trials of the preceding year—doubtless because the low temperature phase had been slightly extended as a precaution against mould wastage—but it was reported to be " incomparably better " than had previously resulted after shipment at 31° F. Fungal rotting due to *Rhizopus* and *Penicillium*, was appreciable in the Kelsey plums ; but enquiries among 300 or so retailers in various parts of the United Kingdom revealed general agreement that any small increase in wastage had been more than counterbalanced by the improved final quality.

After the 1939/40 season the progress of the war prohibited further exports, and they were not resumed until 1946/47. In this year only the three varieties Santa Rosa, Gaviota and Kelsey were shipped, and the dual temperature method was used for all of them. In the 1947/48 season it was used throughout for the much greater number of plum varieties whose export was now permitted. It may be considered now to be well established as the standard method for shipping South African plums to the United Kingdom.

FURTHER POSSIBILITIES.

These investigations have not, of course, exhausted the possibilities of applying a system of modulated temperature control to the overseas carriage of plums. As more data become available it will readily be possible to modify either the initial temperature or the final temperature, or the time at which the transition is made. If the risk of mould damage could be further reduced, by extreme care in packing and handling, by greater stringency in the elimination of slightly blemished fruits, and possibly also by fungicidal treatments, it might well become advantageous to employ a final temperature nearer to 65° than 50° F., and then perhaps to make the transition at a rather later stage of the voyage. In short the one variable of carrying temperature has been replaced by at least two variables of temperature and one of time, and the range of alternatives which are technically practicable is correspondingly widened. Refrigeration may, in this way, be made a more flexible, and therefore a more capable, tool in the business of overseas transport.

What applies to carriage overseas can apply to storage on land, and what applies to temperature may perhaps be extended to other storage variables such as the carbon dioxide content and oxygen content of the storage atmosphere. No innate reason is apparent why the particular combination of storage conditions which is most suited to one phase of the metabolic process in a fruit or vegetable should be equally the most suited to another phase. Existing storage data for most fruits refer to conditions which are held steady during the whole period of test ; but, though scientific experimentation can most conveniently be made under static conditions, it is scarcely more difficult in commercial practice to alter the conditions, should an alteration be desirable, than it is to maintain them constant.

Perhaps the most obvious circumstances in which it may be profitable to investigate modulated temperature control are those connected with the storage of fruits which are liable to low temperature injury. It is noteworthy that the three fruits which play a predominant part in world trade overseas, namely, oranges, bananas and apples, are all thus liable (6). The more inherently perishable the fruit the more acute becomes the problem of how best to take advantage of low temperature in retarding the rate of metabolism and the rate of fungal wastage, without so far disturbing the direction or balance of metabolism that irreparable injury results. At present there are but few data to indicate what periods of storage at various low temperatures can be tolerated by fruits, without injury that is irreversible when the temperature is subsequently raised to another level. It has recently been shown (8) that when English-grown Victoria plums are stored at 31° F. the onset of the damage from internal browning, to which they are very prone after storage for three or four weeks at this temperature, can be greatly postponed if they are transferred for a couple of days to 65° F., at a stage between the fifteenth and twentieth day. The effect can be hypothetically pictured as due to the accumulation at low temperatures of a toxic or inhibiting substance, which can be removed again at a higher temperature if its accumulation has not proceeded too far.

SUMMARY.

1. An account is given of experimental work, carried out in the years 1937 to 1940, which led to the adoption of a "dual temperature" method as standard practice in the refrigerated carriage of South African plums to the United Kingdom.

2. The method is to cool the fruits immediately and rapidly to a temperature as near 31° F. as is practicable, and to carry them on board ship at this temperature for a specified time (usually four to five days), after which the carrying temperature is raised to 45° or to 50 °F., according to the varieties which are being dealt with.

3. More general implications of the method are briefly discussed.

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REVIEWS

CHERRIES. By N. H. Grubb. (London: Crosby Lockwood & Son, Ltd. 1949. Pp. 186 with 12 colour plates and 40 photographs. Price 30s.)

This book marks a definite advance on Bunyard's Handbook of Hardy Fruits, published in 1925, insofar as it includes more descriptions of varieties, a number of photographs of tree forms and fruit shapes, and twelve colour plates of fruits. The photographs of tree form show the trees either in blossom or in leaf, and this rather masks the actual tree shape; on the other hand the colour plates are very good likenesses.

Edward Bunyard claimed to have grown himself nearly all the cherry varieties described in his book, and many of the descriptions of flowers, leaves, shoots and fruits were taken from comparatively young trees in pots. For his descriptions of tree forms Bunyard took most of his notes from established cherry orchards.

Mr. Norman Grubb, in addition to records from his cherry variety collection at East Malling, made careful observations of tree form from cherry orchards in many different parts of the country. It is clear that both these great cherry experts came to regard tree form as being on the whole a better guide to a variety than fruit characters. Bunyard certainly set great store by blossom characters in the identification of cherries, but Grubb, whilst grouping varieties into early, mid-season and late blossoming categories, does not appear to have used actual flower characters as aids to distinguish one variety from another. This might have proved a help in the difficult A, B and C variants of Black Tartarian, and other cherry varieties, as it undoubtedly seems to have been to Crane when trying to separate out the Greengages.

In spite of the many advantages of Grubb's large volume over the small Handbook, it does not by any means supersede Bunyard's little masterpiece, and the two books should always be used together in tracking down cherry varieties. Mr. Grubb has earned the gratitude of everybody connected with cherry-growing by this magnificent piece of detailed systematic pomology. He has refused to be hurried into premature descriptions or to be unduly influenced by what other people think. In Part I Mr. Grubb has given us the benefit of his ripe experience in the planning, planting and management of cherry orchards, and this forms an admirable background for the Classification and Description in Part II.

And now, please will somebody buy a ticket for France, and send Mr. Grubb over there with instructions not to come back until he has solved the mystery of Ste. Lucie and Mahaleb. Mr. Grubb implies in his book that seedling variation may exist in Mahaleb, but that did not deter the workers on St. Julien, Myrobalan, Black Damas and many other plum seedlings from pegging away at them, and separating the sheep from the goats. Nobody seems to have done this for Mahaleb.

N.B.B.

THE PRUNING OF APPLES AND PEARS BY RENEWAL METHODS. By C. R. Thompson. (London: Faber & Faber, Ltd. 1949. Pp. 210, pls. 88, figs. 27. 12s. 6d. net.)

It is now eight years since Mr. Thompson's wartime articles were published. At that time he promised us a pruning manual that would serve as an authoritative basis for the teaching and practice of pruning; many therefore may be disappointed at the title and scope of the present work. The author does not claim that the renewal methods of pruning described in his book are the positive results of research work, but rather that they are based upon deductions from pruning research and the author's own experience at Long Ashton Research Station and latterly as an Advisory Officer in Kent.

The introduction deals with the subject of pruning for the amateur and is followed by a chapter on the functions and scope of pruning. The rest of the work is divided into two main sections dealing separately with the pruning of trees planted for commercial and for intensive culture. Recommendations are made for the treatment of trees that are biennial in fruiting, of neglected and crowded trees and of those which have been head-grafted. Section II covers the pruning by renewal methods of oblique cordons and dwarf pyramids, in addition to a chapter on the reduction of vigour by deferred winter pruning, summer pruning, bark ringing and root pruning. Two appendices, one on pruning costings and the other on the protection of pruning wounds, followed by a glossary of pruning terms, complete the work. Numerous plates and diagrams show the various types of tree, branch and lateral growth, and illustrate such operations as ring pruning, spur reduction, " notching " and " nicking " of buds, dehorning and staggered leader pruning.

The work presents us with two new pruning terms, " bi-lats " and " tri-lats ", for two-year and three-year (or older) laterals respectively. The terms " leader " and " lateral " are adequately described in the glossary, yet frequently throughout the text reference is made to " branch-forming shoots " where " branch-leader " is intended. Similarly " renewal leaders " are defined in the text but are referred to in the diagrams as " replacement leaders ", yet neither appears in the glossary.

Crop figures for trees of Miller's Seedling are given over an eleven-year period in support of pruning treatments applied during the latter four years to control biennial bearing but the age of the trees is not given and, as half the original number of trees were removed one year after the pruning treatments were applied, it is difficult to pass fair judgment on the graph presented.

In the second appendix, on the protection of wounds, tar oil distillate and Stockholm tar are still recommended besides the more satisfactory white lead with linseed oil but grafting wax as an alternative is not mentioned. It is recommended that the wounds of summer-pruned shoots should be covered with paint because of the liability of such shoots to contract perennial canker (*Gloeosporium perennans*); this recommendation suggests a somewhat optimistic view of the time and labour likely to be available at this busy season of the year.

This work should have a wide appeal to pomologists, growers, students and amateurs, but certain points need closer attention in a second edition. Plate 27 is inverted, the last paragraph on p. 46 needs clarification and on p. 148 it should be emphasized that Williams' Bon Chrétien, Dr. Jules Guyot and Marguerite Marillat need to be double-worked on a quince rootstock. The reference on p. 20 to that page seems unnecessary, while the frequent cross references could be reduced in number with advantage. The paper marker, giving the apple varieties under their group headings, which accompanied Mr. Thompson's earlier work, would be appreciated in the present volume, and it would be advantageous if the summaries of pruning treatment at the end of certain chapters could be collected to form an appendix for easy reference.

Readers who have attended Mr. Thompson's pruning demonstrations in the field know him as an able exponent of his art, but his book makes difficult reading—which seems a pity. A.P.P.

GROWTH SUBSTANCES AND THEIR PRACTICAL IMPORTANCE IN HORTICULTURE. By H. L. PEARSE. Technical Communication No. 20 of the Commonwealth Bureau of Horticulture and Plantation Crops. (East Malling: Comm. Bur. Hort. and Plant. Crops. 1949. Pp. 233. Price 12s. 6d.)

Though the hormone concept of plant growth is over eighty years old, its prophetic aptitude was demonstrated by the isolation and identification of the auxin group of hormones less than twenty years ago. One of these substances, hetero-auxin, was a known organic compound, indole-3-acetic acid, and its ready synthesis was directly responsible for one of the most remarkable horticultural developments ever, and one continuously gathering momentum in its application, exercising demands on the plant physiologist unlikely to be met before much more fundamental research can be undertaken.

There were two reasons for this unique course of events. First, the relatively unlimited supplies of indole-3-acetic acid enabled experiments on the external application to plants of a naturally occurring hormone to be carried out in a large number of centres all over the world; second, it initiated chemical synthesis of related compounds thus enabling the study of the relationship between molecular structure and physiological activity. As a result a new field of chemical industry came into being, the production of synthetic plant growth regulators, for that is exactly what these substances are; they regulate and control the course of plant development and there is no part of the plant which cannot be influenced by them.

Even in the first five years after the introduction of synthetic growth substances the number of scientific papers on their effect ran into hundreds, and the review of these by Dr. Pearse in 1939 (Tech. Comm. 12) commanded our respect and admiration. But his task nearly ten years later was a far more difficult one and for this reason the present review is even more welcome and appreciated. Some 1,500 papers are now referred to, drawn from a widely scattered literature, and presented in co-ordinated fashion with critical and authoritative comment.

The pattern of the book resembles that of its predecessor, with pride of place again going to root stimulation of cuttings, the results with many hundreds of species and varieties being in tabular form. Dr. Pearse is to be particularly congratulated on his treatment of this subject. Next comes a section on the growth substance treatment of seed and seedling, another on the treatment of growing plants passing to parthenocarpy, hormonal herbicides, inhibition effects, and finally a section on miscellaneous uses of growth substances. The control of pre-harvest fruit drop by growth substances is deliberately omitted, as this subject has been treated in detail elsewhere (Tech. Comm. 18). The only criticism is that no index is included, though a very full list of contents helps to some extent.

Whether to the practical horticulturist anxious for technical information and description of methods, to the student of botany, horticulture or plant physiology, or to the research worker with his great problem of being kept informed of the literature, this latest technical communication of the Commonwealth Bureau of Horticulture and Plantation Crops will come as a valuable contribution to horticultural science, enabling the reader to exercise to maximum extent his own judgment in matters not always beyond the bounds of controversy.

Meanwhile the flood of papers on the influence of synthetic plant growth regulators flows with increasing power and while we do not envy Dr. Pearse when the time arrives for a third review, we are sure that he, more than anyone else, will be the person to undertake it.

The identity of heteroauxin was the stimulus to these great developments. At the time it was thought that this substance did not occur in higher plants, only in fungi, hence its name; but it is now established that in all probability this is the most widely occurring auxin of the whole plant kingdom, the Phanerogams included. This confers an even greater importance on the studies of the effects on the plant of the original synthetic plant growth regulator and natural auxin indole-3-acetic acid.

E.S.J.H.

FRUIT CULTURE. By A. H. Hoare. (Edinburgh: Thomas Nelson & Sons, Ltd. 1949. Pp. 347, pl. 15. Price 12s. 6d.)

The scope of this book is vast ; written mainly for the commercial fruitgrower, it describes the characteristics and culture of some forty different fruits, from apples to oranges, including salad fruits such as tomatoes and cucumbers. The first part of the book surveys the nutritive value of fruit, and areas of production, and then devotes chapters to the general principles governing propagation, selection of site and all the routine involved in establishing and maintaining fruit plantations. The second part deals with each fruit in turn, presenting botanical data before giving lists of recommended varieties, rootstocks, planting arrangements, marketing and economics. In this part also there are chapters devoted to the culture of fruit for canning and in home gardens, and a chapter giving brief descriptions of the chief pests and diseases. Appendices outline the management of special propagation nurseries for raising plants of strawberries and raspberries, and give a table of numbers of trees per acre for different spacings. The author has a fluent, easily read style and the book is well-planned and attractive but on the whole the treatment is superficial and too little detail is given to make it of much value to research workers or experienced fruitgrowers. Some of the advice is inaccurate or out-of-date, obviously due in certain cases to the two years' delay in publication ; the possibly harmful effect of some of this advice is offset by the lists of references for further reading at the end of each chapter. This is a book for the newcomer to the industry who wishes to survey the whole field of fruitgrowing and should serve as a background to more detailed study in other works once the field has been narrowed to include only certain fruits.

H.B.S.M.

THE QUARTER-ACRE GARDEN. By George Whitehead. (London: Pilot Press. 1949. Pp. 124, 8 pl. Price 6s. 6d.)

A book for garden beginners, who are likely to be fascinated by the plans and suggestions on layout in the earlier chapters but to graduate quickly from the brief accounts of flower, fruit and vegetable cultivation in the later chapters to more advanced texts.

E.E.C.

CHRYSANTHEMUMS. Bulletin 92 of the Ministry of Agriculture and Fisheries. (London: H.M. Stationery Office. 1948. Pp. 43+iv. Frontispiece and 12 pls. Price 1s. 6d.)

This publication, a revision of a bulletin first published in 1936, is designed to assist growers interested in the production of Chrysanthemum flowers for market.

It deals with the plant, classification and choice of varieties, propagation, cultivation both in the open and under glass, and preparation for market. The bulletin is well illustrated, and very cheap at its price.

E.E.C.

NATIONAL FRUIT TRIALS, 1945-47. By J. M. S. Potter. (London: Royal Horticultural Society. 1948. Pp. 32. Price 2s.)

This is a continuation of the Report of the National Fruit Trials for the years 1921-1944 made by the Chief Officer, Mr. J. M. S. Potter, and published in 1945, and covers the years 1945-1947. Each year is dealt with separately.

Frosts interfered with the trials in 1945 and 1946, especially in the former year, but exceptionally good crops were obtained in 1947.

Many new varieties of fruits are described and attention is drawn to those thought likely to suit commercial or domestic producers.

Some valuable notes by Dr. L. E. Campbell, Director of the British Food Manufacturing Industries Research Association, on varieties of soft fruits in relation to jam manufacture, are included in an Appendix.

The reports of these trials contain much of interest and should prove of great assistance to growers and others who are searching for new varieties to try out.

Especially valuable is the inclusion of information as it becomes available on such points as susceptibility to disease; suitability or otherwise of soft fruits for canning, jam, or dessert; and preference of a variety for any special conditions or locality.

A.C.P.

THE CITRUS INDUSTRY. Vol. II. THE PRODUCTION OF THE CROP. Edited by L. D. Batchelor and H. J. Webber. (Berkeley and Los Angeles: University of California Press. Agents in Great Britain and Ireland: Cambridge University Press. 1949. Pp. 933. Price 55s.)

This long-awaited second volume of The Citrus Industry appears five years after Vol. I, which was devoted to the history, botany and breeding of citrus crops. It is an outstanding contribution to citrus literature being in the form of a symposium by no less than sixteen authors, each of whom writes with specialist knowledge based on experimental work—an admirable method of presentation, but one which leads to a certain amount of overlapping and, sometimes, to different interpretations of the same phenomena. The editors' plan has been to present the subject in such a way as to be applicable anywhere in the citrus-growing world, but since the text has been prepared by Californian investigators it is inevitable that Californian practice should loom largest. With this the foreign reader will find no fault, knowing the high stage of advance achieved by the citrus industry of that State. The great scope of the book can best be illustrated by quoting the following chapter heads into which it is divided; nursery methods, the character and reaction of rootstocks, choice of rootstocks, selection of orchard site, planning and planting, cultivation, principles and methods of fertilizing [manuring], cover crops and green manures, pruning, irrigation, diseases and their control, biological control of insect pests, insects and mites, quarantine services, injury by rodents, frost protection, effect of freezes and treatment of frost-injured trees. The subject of rootstocks has received particular attention. This is very appropriate at the present time when the relation of rootstocks to the spread or prevention of various virus and fungus diseases has brought about a keener interest in rootstock problems. The chapters on choice of rootstocks, their characters and reactions, incorporate an account of the main results and conclusions from over 25 years' work at the Californian Citrus Experiment Station—probably the most extensive and longest-continued experiments ever conducted with citrus rootstocks. Chapter XI, Diseases and their Control, by H. S. Fawcett and L. J. Klotz, is a summary of the much fuller information given by the same authors in their book, *Citrus Diseases and their Control* (McGraw-Hill, New York). Similarly the subject of Chapter XIV, Insects and Mites and their Control, by A. M. Boyce, is more fully treated in *Insects of Citrus and other Sub-Tropical Fruits* by H. L. Quayle (Comstock Pub. Co., New York).

This second volume of The Citrus Industry, a truly worthy successor to the first, can be unhesitatingly recommended to research workers, students and growers, but the last-named may find that some of the explanations given are too technical to be properly understood. We look forward to Vol. III, Harvesting, Marketing and Utilization, which will complete the trilogy. A. G. G. Hill.

TOMATO ROOT INJURY DUE TO CHESHUNT COMPOUND

By H. H. GLASSCOCK and W. DERMOTT
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IN the course of advisory work, many instances have been met in which roots of tomato plants have shown dead and discoloured tissue, yet no associated parasite could be found. It has sometimes been possible to trace the damage to treatment such as the application of an artificial fertilizer in quantities sufficient to " scorch " the roots but often the cause has remained unexplained. In discussing cultural treatments, the grower has not infrequently disclosed that Cheshunt Compound had been applied to the soil as a protection against *Phytophthora* root rots. It has been further ascertained that a number of growers hold the view that Cheshunt Compound, when applied with a frequency greater than that recommended, cannot cause damage to roots, and this belief has sometimes led to the indiscriminate use of the fungicide from the time of sowing the seeds until the plants are well established.

The writers, considering that at least some unexplained root damage might be the result of the application of Cheshunt Compound either too frequently or in excessive concentration, decided to test these views and experiments were carried out in 1947 and 1948.

EXPERIMENT OF 1947.

In 1947, seeds of the variety Potentate were sown in two boxes containing a compost made to the John Innes formula (J.I.S.). Both boxes were watered in the normal manner until the seed leaves had unfolded, when watering was continued with one box (control) and an equal quantity of Cheshunt Compound solution was applied at the same intervals to the other. The compound used was a proprietary preparation and the solution made up to the recommended concentration (1 oz. in 2 gallons of water).

The first application of Cheshunt Compound (250 ml.) was made on 8th March, and others on 12th, 15th and 18th respectively. On 20th March, 12 plants from the treated box and 12 plants from the control box were transplanted into pots containing compost made to the John Innes formula (J.I.P.1). The loam constituent, with pH 7·1, was from the same source as that used in the seed compost.

Up to the time of transplanting, seedlings had been removed at intervals and the roots examined but no damage had been observed. The only marked difference between the treated and control plants was the darker green colour of the former. This had been discernible on the fourth day after the first application of Cheshunt Compound and was the expected reaction attributable to the uptake of nitrogen contained in it.

Normal watering was continued with the control plants and an equal volume of the Cheshunt Compound solution applied to the others, the first application (25 ml. to each pot) being given on 21st March (the day after potting). Eight further applications were made on the 24th and 28th March, 1st, 6th, 10th, 14th, 19th and 24th April respectively. A volume of 30 ml. was given to each pot at each application.

Two treated plants and two control plants were examined at weekly intervals. Rate of growth, condition of roots and general appearance were compared. Throughout the period the foliage of the treated plants was of a darker green than that of the control plants. Relatively less growth of the treated plants was first noted on 10th April, when a total of eight applications of Cheshunt Compound had been given. The tips of many of the rootlets, particularly the upper ones, were discoloured brown and had a " burnt " appearance; it was also evident that the roots as a whole had made less growth. The control plants had produced normal growth. On 19th April, the 42nd day after the unfolding of the seed leaves and after the tenth application of Cheshunt Compound, measurement showed that the height above soil level of the control plants ranged from 7 in. to 7½ in. while that of the treated plants was from 5½ in. to 6 in. At this stage, treatment was discontinued.

From this experiment it was clearly indicated that damage to young plants was possible by the continued application of Cheshunt Compound of recommended concentration.

EXPERIMENT OF 1948.

It was decided to continue the investigation using " home-made " Cheshunt Compound instead of a proprietary preparation and also to compare the effects of the treatment with composts containing loam with acid and alkaline reactions respectively. In addition, the effect of Cheshunt Compound of double and triple strength was tested.

The Cheshunt Compound was prepared by mixing 2 parts by weight of finely ground copper sulphate with 11 parts by weight of finely ground ammonium carbonate. This mixture was stored in a tightly stoppered jar for at least 24 hours before the resultant compound was used.

On 1st March, tomato seeds of the variety Potentate were sown in three boxes. Alkaline loam (pH 7·1) was used in the compost (J.I.S.) contained in two boxes and acid loam (pH 5·1) in the compost contained in the third. Finely ground calcium carbonate had been added to the acid sample in prescribed quantity (1½ oz. per bushel).

From the sowing of the seeds until potting fifteen days later, water was applied to one of the boxes containing alkaline-loam compost and equal quantities of Cheshunt Compound to the other and to the acid-loam compost. Altogether three applications, each of 500 ml., were made on 1st, 5th and 11th March respectively.

By 13th March there were marked differences in the size and appearance of the seedlings. Those in alkaline-loam compost, which had been treated with water, were light green in colour and the seed leaves had a span of 2¼ in. to 2½ in. from tip to tip. Those in the acid-loam compost and treated with Cheshunt Compound were of about the same measurement, but were darker green in colour. The seedlings in the box of alkaline-loam compost, treated with the Compound, were also dark green, but with a span of only 1 in. to 1¾ in. No damage to the roots was observed.

On 16th March, 24 seedlings from the watered alkaline-loam compost were transplanted into individual pots containing compost (J.I.P.1) made with alkaline loam and normal watering was continued. Similarly, 21 seedlings from the alkaline-loam compost which had been treated with Cheshunt Compound were potted and applications of a solution of the Compound continued.

Seedlings from the acid-loam compost were potted in compost (J.I.P.1) containing acid loam but, of a total of 12 seedlings, six were watered normally while Cheshunt Compound treatment was continued with the other six. The first application was given when the seedlings were potted and subsequent applications were made at intervals of about three days. For each of the first seven applications, 100 ml. per pot was required and for the last three, 200 ml.

In addition to frequent observations made on the foliage, plants were removed each few days from the watered and from the alkaline-loam compost and the roots examined.

A summary of the observations after potting is given below in tabular form :—

	Alkaline-loam Compost.		Acid-loam Compost.	
	Watered.	Cheshunt Compound treated.	Watered.	Cheshunt Compound treated.
19th March .. (3 days after potting)	Healthy, vigorous plants.	Plants recovering from check as seedlings and nearly same size as control.	Equal in size to to control. Slightly chlorotic.	Slightly smaller than control. Foliage dark green.
24th March .. (8 days after potting)	Healthy vigorous plants.	Growth again visibly checked. Tips of rootlets brown.	As control.	As control. Foliage darker green.
1st April .. .. (16 days after potting)	Healthy vigorous plants.	Growth markedly checked. Tips of rootlets brown.	As control.	As control. Foliage darker green.
19th April .. (34 days after potting)	Healthy vigorous plants. Stem 12″ long.	Stunted. Stems 3″-9″ long. Root development inhibited. Rootlets brown.	As control.	As control. Foliage darker green.

At the end of the experiment, samples of compost from all the main treatments were analysed by the modified Morgan Method (Tinsley and Pizer, 1945 and 1946) for available phosphate and potash and the reaction tested by glass electrode as follows :

Treatment.	pH.	" Available " Phosphate.	" Available " Potash.
Alkaline-loam, watered ..	7·1	Very High	Very High
Alkaline-loam, Cheshunt Compound	6·6	Very High	Very High
Acid-loam, watered	5·0	High	Very High
Acid-loam, Cheshunt Compound	4·6	High	Very High

A few of the treated seedlings which had been potted in alkaline-loam compost were given doses of Cheshunt Compound at twice the normal concentration and others at treble strength. Inhibition of growth was more severe under both treatments than with Cheshunt Compound at normal strength. By 7th April, plants under treatment with treble concentration were not only very stunted, but, of four plants treated, two were dying and root scorching was severe. These higher concentrations of Cheshunt Compound were not tested in conjunction with acid-loam compost.

CONCLUSIONS.

Under the conditions of the experiment in 1947, it was shown that the use of Cheshunt Compound solution instead of water resulted in root damage and showed a definite check to the growth of tomato plants after eight applications during a period of 30 days and that these effects were increased by further applications.

In 1948 the experiment was repeated in a modified form and it was found that when Cheshunt Compound solution was applied from the time of sowing seed, development of the seedlings was markedly inhibited within 12 days. There was a short recovery after potting, but within 8 days a check to development was evident and 34 days after potting serious stunting was seen and root damage was marked (Fig. 1).

In the experiments where damage occurred the loam component of the compost was slightly alkaline (pH 7·1). In a parallel experiment where acid loam (pH 5·1) was used and equal quantities of Cheshunt Compound applied, root damage and growth inhibition were negligible.

The frequency with which Cheshunt Compound was used in the experiments may have been in excess of that likely to be found in practice, but continued application over long or short periods has been admitted by some growers and could account for root damage under certain conditions.

The influence of the different loam components on root damage may have been due to differences in reaction and content of " free " calcium carbonate, but account must be taken of the possible influence of other properties. If reaction is responsible, then liberation of ammonia by free calcium carbonate in the alkaline sample is a likely explanation.

The writers wish to emphasize that the account which they have given of root damage following the use of Cheshunt Compound refers only to applications which were excessive in number or in strength of the solution. Cheshunt Compound has been for many years a most valuable fungicide for use by tomato growers when an epidemic of " Damping Off " caused by *Phytophthora* sp. has occurred in batches of seedlings or young plants ; the spread of the disease has often been checked by the judicious " watering " of the plants with a solution of Cheshunt Compound. Also, if there has been any doubt about the efficiency of measures of hygiene taken between crops, then boxes and pots may be treated before and after planting. The soil in a house may be treated first, then planted and treated again without risk of significant damage to the plants.

Thanks are given to Professor H. W. Miles, of Wye College, for providing glasshouse facilities without which the work could not have been done.

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Tomato Root Injury due to Cheshunt Compound.
By H. H. Glasscock and W. Dermott.

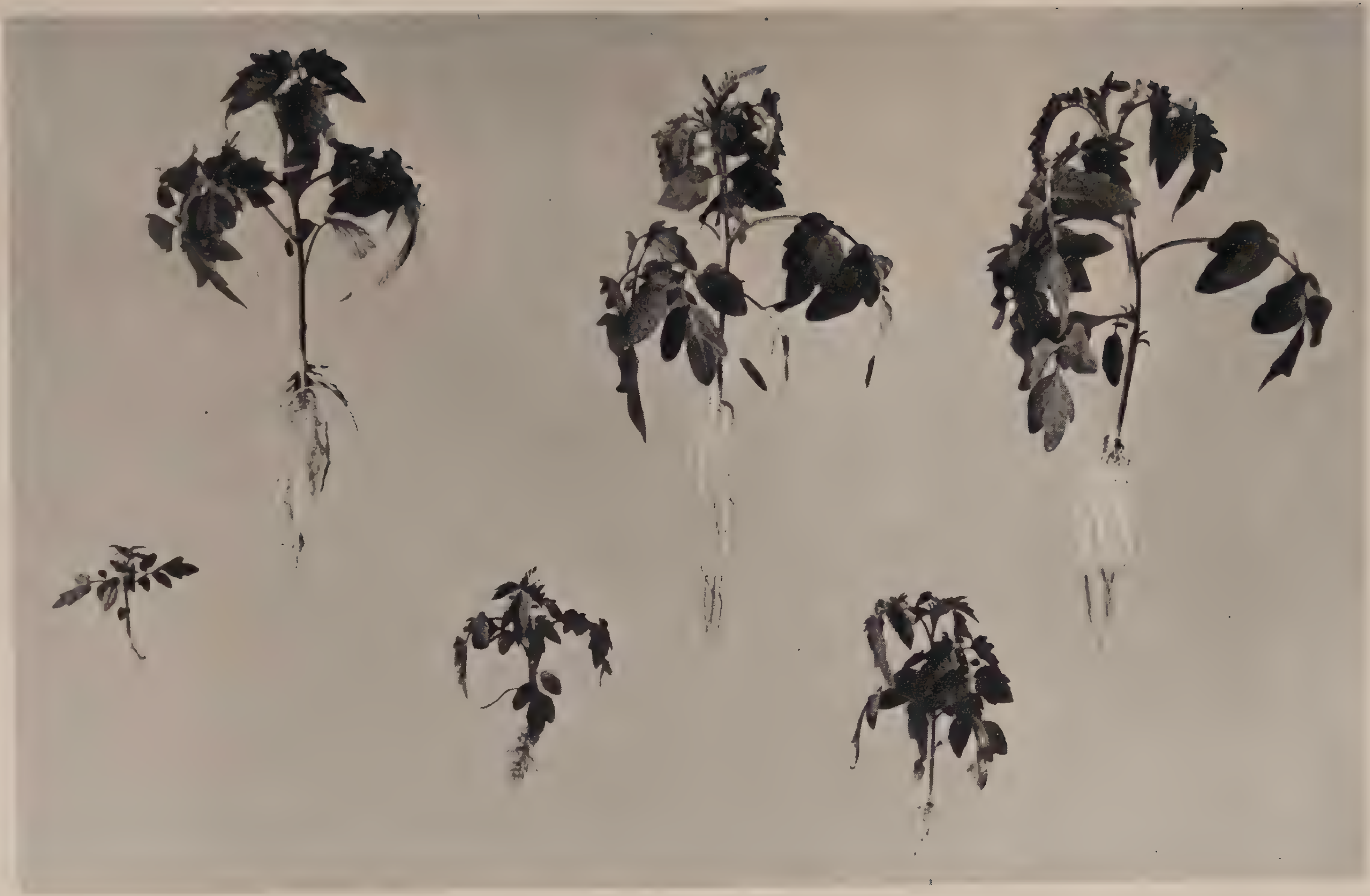

Fig. 1.

Excessive application of Cheshunt Compound to tomato plants. All plants 34th day after potting. Top Row: Normal watering. Bottom Row: Cheshunt Compound used instead of water.

THE POLLINATION OF CACAO IN THE GOLD COAST

By **A. F. POSNETTE**
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The writer's earlier investigations into the natural pollination of cacao (Posnette, 1942a, 1942b), proved that at least half the pollination at Tafo (Gold Coast) was effected by flying insects, the identity of which was then unknown. These have now been identified and the degree of cross-pollination done by them has been investigated during the past five years. The pollination of cacao is important from two considerations: firstly, we must know to what extent clonal plots must be isolated if the male parent of "clonal seed" is to be known, and secondly we must consider what effect insecticidal measures against pests will have on the pollinating insects.

Little importance was attached to the pollinating agents of cacao until self-incompatibility was shown by Voelcker (1937, 1938a) to be a permanent state of a high proportion of cacao trees in Trinidad, including many of the clones selected for high yield. The literature on pollination of cacao has been reviewed by Voelcker (1938b) and Posnette (1944), and the salient points will be recapitulated in this paper only where they are relevant to the particular aspect of the investigation being described.

MECHANISM OF POLLINATION.

Following the work of Billes (1941), the writer (Posnette, 1944) investigated the pollination of cacao flowers by Ceratopogonid midges in Trinidad, and showed that at least 75 per cent. of the flower-setting followed pollination by these insects, two species of which have since been identified by Dr. J. W. S. Macfie (1944) as *Forcipomyia quasi-ingrami* Macfie and *Lasiohelea nana* Macfie. This author mentions that these species have also been collected in Brazil and Costa Rica.

Microscopical examination of pollinated flowers on the Gold Coast revealed the clusters of pollen grains characteristic of pollination by Ceratopogonids (Fig. 1). The insects were collected by trapping in cacao flowers at Tafo and were determined by Macfie as *Forcipomyia ingrami* Carter, *F. ashantii* Ingram and Macfie, and *Lasiohelea litoraurea* Ingram and Macfie.

These insects escaped detection for so long on account of their small size and the difficulty of trapping them, due to the relatively short time spent in any one flower and because they are easily disturbed and leave the flower rapidly. They are almost impossible to see in flight unless they are carrying pollen but as they frequently collect a ball of pollen nearly half as large as themselves, their progress from flower to flower has been followed by watching the white speck of pollen as it is carried through the air. No differences in behaviour have been noticed between the pollinating Ceratopogonids in the Gold Coast and those in Trinidad. The cacao flower is ideally constructed for pollination by these insects; they appear to be attracted by the deeply-pigmented tissue of the staminodes which surround the style and of the "guide lines" which run inside the petal hood from the base of the ovary to the anther (Figs. 2 and 3). Whether the midges actually feed on this tissue has not been determined but they probe with their mouthparts as though they were feeding.

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The insect usually alights on a staminode, and as it walks up the inner side, the thorax brushes against the stigma or style and the pollen is removed either as a cluster of grains or as a smear. Afterwards, the insect enters the petal hood and follows the " guide lines ", so that at the apex of the hood the bristles of the thorax collect a ball of the slightly sticky pollen from the anther. When the insect flies to another flower, pollination is effected as it walks up the staminode. Most of the insects which have been watched flew from one flower to another on the same tree, but flight from one tree to another has been observed on two occasions and probably the majority of those which eventually flew out of sight, went to other trees. It is easy to understand that flowers often receive pollen from several different trees, either by a single insect carrying mixed pollen or by different insects visiting the same flower. This occurrence was postulated by Voelcker (1938c) to account for non-Mendelian ratios in the segregation of genetical characters.

Microscopical examination of pollinated flowers gave corroborative evidence that Ceratopogonids were responsible for much of the pollination. Firstly, fine hairs or bristles of the same shape and size as those on the wings and thorax of *Forcipomiya* spp. were sometimes found on the style or in the pollen clusters (Fig. 1). These bristles are easily detached and many have been lost in the mounting of the insect shown in Fig. 4. Secondly, the pollen clusters or smears on the style were opposite the staminodes on which the insects walk, not between them as would be expected if the pollen were shaken or blown from the anthers onto the style. This is difficult to accomplish owing to the stickiness of the pollen and the hairs fringing the staminodes and could rarely happen in the sheltered conditions under which cacao is usually grown.

INCIDENCE OF CROSS-POLLINATION.

Genetic factors.

Two inherited characters have been used to determine the incidence of cross-pollination at Tafo—" albinoism " (complete absence of chlorophyll) first discovered by the writer at Tafo in 1944, and the axil spot used by Harland and Frechville (1927) and Voelcker (1938c).

Albinoism is a recessive character ; the degree of cross-pollination occurring on a single tree with this factor can be determined exactly, since only self-pollinated pods will produce albino seedlings. Axil spot in the form of red pigment at the junction of petiole and stem is linked with red pod and is visible in seedlings immediately after germination. Harland and Frechville found in Trinidad that axil spot is controlled by two complementary dominant factors and could, therefore, appear in the progeny of a green-pod × green-pod cross. The absence of red pods from Amazon cacao in general and from the West African Amelonado form in particular, shows that one (or both) of these complementary factors is absent. Neither the writer nor Voelcker (1940) has ever obtained progeny with axil spot from crossing two green-podded trees, and the character can, therefore, be used for tracing the distance over which pollen is carried from a red-podded tree into surrounding Amelonado cacao with green pods. Only if a green-podded tree is surrounded by red-podded trees can this character be used to determine accurately the degree of cross-pollination.

Albino seedlings.

Owing to the presence of self-sown albino seedlings, a tree with a recessive albino gene was discovered among the cacao on Tafo station in 1944 and hand-pollination was carried out in 1945 to determine the genetic system involved. When this tree (No. H 52·1) was self-pollinated by hand, the seedlings from 20 pods segregated into 606 normal and 172 albino, but, when cross-pollinated with an adjacent tree, 55 pods gave 2,050 normal seedlings and no albinos. It is clear that a single recessive gene is involved; since the albino seedlings die when the reserves of the cotyledons are exhausted, the homozygous constitution is lethal. None of the surrounding trees produced albino seedlings.

All the naturally pollinated pods of the " albino tree " maturing in 1946, 1947 and 1948 were planted and the number of normal and albino seedlings from each pod were recorded. As shown in Table I, the percentage of pods resulting from cross-pollination varied from 43 per cent. in 1946 to 24 per cent. in 1948; as the number of pods harvested each year also varied greatly, this difference in cross-pollination may be caused more by the size of the crop than by climatic changes from one year to another. However, there is evidence from the proportion of normal to albino seedlings that cross-pollinating insects were more prevalent in 1946. From Table II it can be seen that in 1947 and 1948 the self-pollinated pods segregated 3 normal to 1 albino seedling, but in 1946 they segregated 8 normal to 1 albino, indicating that some of the flowers were pollinated by both self and cross pollen, an occurrence more likely to happen the more abundant the pollinating agents.

TABLE I.

Natural pollination on " albino " tree.

Year.	Total pods.	Selfed.	Crossed.	% Crossed.
1946	14	8	6	43
1947	3	2	1	33
1948	56	44	12	24

TABLE II.

Segregation from selfed pods in Table I.

Year.	Seedlings.		% Albino.
	Normal.	Albino.	
1946	342	42	11
1947	47	14	23
1948	1,072	358	25

Axil spot.

Although several red-podded trees surrounded by green-podded trees were known since 1942, and used for estimating the extent of cross-pollination, it was not until 1948 that I found green-podded trees each entirely surrounded by trees with red pods. The crop from these five green-podded trees was planted in pod rows and the presence of seedlings with axil spot used to determine the degree of cross-pollination. The number of pods harvested is insufficient to consider the trees individually, but as they are growing on the same acre, the total of 90 pods with 18 per cent. cross-pollinated is probably a reasonable estimate for that plot (Table III). It is interesting to note that cross-pollination was of the same order as the 23 per cent. recorded during the same year for the albino trees growing about one mile away.

TABLE III.

Natural pollination on green-podded trees surrounded by red-podded trees (1948).

Tree No.	Total pods.	Total crossed.	Segregation from crossed pods.		
			Axil Spot.		% with axil spot.
			Absent.	Present.	
1	25	5	124	68	35
2	3	2	58	36	38
3	13	4	81	77	49
4	25	2	33	12	27
5	10	1	31	17	35
Totals ..	76	14	327	210	
%		18		39	

Seasonal effects.

If cross- and self-pollination were effected by different species of insects, as postulated by Cope (1940), seasonal differences in the incidence of cross-pollination might be expected and, if this were so, it might be possible to predict that all pods harvested at a certain time of the year would be self-pollinated.

An examination of the harvesting dates of the pods in these investigations shows that although in any one year cross-pollination was more prevalent in one particular month than in others, the month varied from year to year and some cross-pollination occurred whenever flowers were set.

THE EXTENT OF CROSS-POLLINATION.

Investigations into the distance over which pollen is carried were commenced in 1942 at Aburi and continued on three different plots at Tafo until 1947.

The Aburi plot consisted of a single red-podded tree in an isolated plot of trees with green pods. Hand pollination showed that the "green" trees produced "green" seedlings with no axil spot pigmentation when self-pollinated, but, if crossed with pollen from the "red" tree, seedlings segregated in the proportion of 1 red to 3 green (88 red : 261 green).

The naturally pollinated seeds from the first two rows of trees surrounding the "red" tree were sown in pod rows and the pods resulting from crossing by pollen from the "red" trees were detected by the presence of seedlings with axil spot. In the 1942 crop, only 2 out of 99 pods from trees in the first row were crossed by pollen from the "red" tree; none of the 89 pods maturing on second row trees gave seedlings with axil spot. In 1943, only 14 pods were obtained from trees in the first row and none was crossed with pollen from the "red" tree but 1 pod out of 69 from the second row was so crossed.

Hand cross-pollination between the red- and the green-podded trees at Tafo was done in 1943. From one plot, the seed from 28 pods segregated into 398 seedlings with axil spot and 435 without ; from another plot, 380 seedlings with, and 489 without axil spot segregated from 32 crossed pods—approximately a 7 : 9 ratio.

The three Tafo plots are more complex than that at Aburi because the cacao is irregularly spaced and not planted in lines. Each plot consists of two red-podded trees separated by one green-podded tree and entirely surrounded by other green-podded trees. In Table IV, the trees are arranged in two groups. " First ring "

TABLE IV.

Natural pollination on green-podded trees surrounding red trees.

1st Ring Trees.			2nd Ring Trees		
Tree No.	Total pods 1943-47.	Pods crossed by red tree.	Tree No.	Total pods 1943-47.	Pods crossed by red tree.
E54/45	111		E54/18	28	
46	89		19	40	1
47	55		20	25	
48	118	2	43	79	
49	80	1	44	31	
50	26	3	75	62	
76	38		77	19	
78	66	1	110	58	
79	75	1	E62/35	68	1
81	87	1	62	32	
82	61		91	41	
83	68	1	94	24	
109	62	1			
E62/92	80				
93	65	1			
15 trees	1,081	12	12 trees	507	2
G83/94	32		G83/90	51	1
96	33		100	26	
99	34		129	13	
131	4		130	17	
132	10		144	8	1
133	12		145	16	
135	34	1			
136	5				
141	5				
143	9	1			
10 trees	178	2	6 trees	131	2
G84/14	32		G84/20	31	
21	31		35	101	2
23	13		43	105	1
25	42	2	44	69	
36	21		45	71	2
38	64		50	4	
40	50	1	51	11	
42	46		54	7	
52	13		55	17	
53	29	1			
10 trees	341	4	9 trees	416	5

trees are those without another tree between them and a " red " tree ; " second ring " trees are those with one tree separating them from a " red " tree but the distance apart is variable. A few trees have been lost because of swollen shoot disease and the fall of a shade tree, so that although the total pods harvested between 1943 and 1947 is given for each tree, this does not represent five years crop in every case.

Each " red " tree in plot E54 had eight first ring trees, the single tree between them being shared. If pollination were confined to adjacent trees, it might be assumed that each tree would derive one-eighth of its cross pollen from each of its immediate neighbours but clearly a proportion of cross pollen comes from trees in the second ring, so that the " red " tree must be expected to provide less than one-eighth of their total cross pollen to each of the first ring trees. About 1 per cent. of the total pods from the first ring trees had been pollinated by " red " pollen and, therefore, it might be deduced that between 8 and 12 per cent. of all their pods resulted from cross-pollination, a much lower proportion than in the experiments in which cross-pollination was determined directly.

This low degree of cross-pollination reduces the value of the results for the estimation of the distance over which pollen was carried. From the E54 plot figure in Table IV, however, it is clear that pollination between trees two rows apart was much less than between adjacent trees, although there is no confirmation of this from the other two plots on which most of the trees were younger and the yields consequently lower than on plot E54. In plot G84, for instance, 5 pods crossed by " red " pollen were obtained from the second ring trees, compared with only 4 pods from the first ring but it is important to note that all 5 pods were from the plot's three highest-yielding trees on which, presumably, the chances would be greatest not only of cross-pollination occurring, but of the resulting sets surviving cherelle wilt and reaching maturity (Humphries, 1943).

DOUBLE POLLINATION.

Voelcker (1938c) gave evidence that in Trinidad different seedlings from the same pod may have different male parents. Both in Trinidad and in the Gold Coast the writer has examined many flowers which had been pollinated more than once as shown by the presence of several pollen clusters on a single style. With the object of obtaining further evidence on the feasibility of mixed fertilization occurring in nature and to ascertain how long flowers must be protected after hand-pollination, experiments on double pollination were carried out.

Flowers on self-compatible green-podded trees were covered before opening and for 24 hours after pollination. It was known that if these flowers were self-fertilized none of their progeny would have axil spots and, if crossed with a red-podded tree, the progeny would be mixed in the proportion of 9 seedlings without to 7 with axil spot. The flowers were pollinated by hand, either first with their own pollen and then with pollen from the red-podded tree after a certain interval of time, or, vice versa. An abundance of pollen was applied each time to the stigma and distal portion of the style.

The figures in Table V indicate that the pollen from the red-podded tree (i.e. cross-pollination) was more effective in fertilization than self pollen. When self pollen was applied first, cross pollen effected fertilization of the majority of the ovules when applied 2 hours later (44 per cent. seedlings with axil spot would be expected if only the pollen from the red tree were effective). The proportion of ovules fertilized by the cross pollen is negligible six hours after selfing ; by this time, according to Cheesman (1927), fertilization by the self pollen should be complete but it is interesting to note that some fertilization by cross pollen occurred 12 hours after selfing.

When cross pollination was done first, self pollen apparently effected some fertilization when applied up to 3 hours later. After this time the variation about the genetical ratio renders the influence of self pollen more difficult to estimate than that of the cross pollen carrying the dominant factor but it appears probable that the self pollen effected little or no fertilization when applied six hours after the cross pollen.

The two facts which this experiment demonstrated are that two or more male parents can be concerned with the progeny in a single pod, and that it is necessary to keep flowers covered for at least 12 hours after controlled pollination to ensure that no pollen contamination will affect the progeny.

TABLE V.

Progeny from double pollination.

Time interval, hours.	Crossed first, selfed later.				Selfed first, crossed later.			
	No. of pods.	Seedling spot.		% with spot.	No. of pods.	Seedling spot.		% with spot.
		Absent.	Present.			Absent.	Present.	
¼ - ½	4	117	23	18	6	151	30	17
1	6	127	12	9	3	50	31	38
2	6	132	43	25	2	35	20	36
3	3	90	22	20	5	87	10	10
4	4	62	38	38	4	134	18	12
5	2	59	7	11	3	88	10	10
6	3	65	31	32	12	297	29	9
9	2	46	30	40	4	122	—	—
12	4	107	42	28	8	244	6	2
17	1	10	5	33	1	32	—	—

DISCUSSION.

A knowledge of the insects involved and of their behaviour in pollination is essential to a rational policy of clonal seed production. Muzik (1948) has pointed out that the pollinating agents of *Hevea* rubber trees are unknown and that seed-setting in clonal plots is scanty except where different clones are adjacent. With self-incompatible clones, on which all setting must be due to cross-pollination, it is relatively easy to determine the optimum size of clonal plots designed for seed distribution from the gradient in setting as the distance from the pollinating clone increases. With self-compatible clones, it is impossible to tell which pods are the result of self- and which of cross-pollination unless some genetical factor is present which will express itself in the seedling progeny immediately after germination. Even when such a factor is present, a special arrangement of the plots may be required if it is intended to distinguish selfed from crossed progeny.

It has been the Institute's policy to develop clones with high-yielding seedling progeny (Posnette, 1943). " Clonal seed " would be distributed from these clones and the design of clonal blocks must now be considered so that pollination can be controlled.

From experiments in Nigeria, Voelcker (1940) concluded that about 25 per cent. of the flowers on self-compatible trees were cross-pollinated and that pollen was unlikely to be carried beyond the immediately neighbouring trees. The results of the experiments described in this paper are in general agreement, which is perhaps surprising when the ecological differences between the cacao plots at Ibadan and at Tafo are considered. At Tafo, guard rows between plots should consist of at least two rows of trees since pollination between trees two rows apart occurred with sufficient frequency to cause serious discrepancies in the progeny. The effect of this pollen contamination would be inversely proportional to plot size, however, and if seed were distributed from 400 trees on a square plot of one acre, with a one-tree guard row, the small proportion of illegitimate progeny might be ignored in preference to reducing the seed available by 19 per cent. (i.e. that from 76 trees in the outside row).

Although it seems probable that pollen is very rarely carried to trees more than two rows distant, there is no evidence from which to determine the maximum distance over which pollen is carried. Circumstantial evidence from clonal plots of white-seeded cacao in Trinidad suggests that pollen may be carried as far as 50 yards, but it is possible that flowers produced on the rootstocks were responsible for pollen contamination. A plot has now been planted at Tafo consisting of 36 budded trees of the " albino clone ", arranged in a 6 × 6 square surrounded by two rows of normal seedlings and this should provide further evidence on the degree and extent of cross-pollination.

If self-incompatible clones are used, it would appear at present that pollinator trees should be placed not less frequently than in every third stand in every third row so that each self-incompatible tree is adjacent to a pollinator tree. In Trinitario cacao the pollinator trees would have to be self-compatible and so their crop would consist of both selfed and crossed pods and would, therefore, be useless for distribution. In Upper Amazon cacao, on the other hand, in which cross-compatibility between self-incompatible types is common (Posnette, 1945) the crop on the pollinator trees would consist of the reciprocal cross and would probably be genetically identical with that on seed-supplier trees. In such a case the decision as to which clone should be used as seed supplier and which as pollinator rests on which is the higher yielder and, if there were little difference in yield, it would be advantageous to plant equal numbers of each clone, arranged in alternate rows, so that the maximum quantity of cross pollen would be available to each tree. This is not a hypothetical case, as the highest-yielding progeny in the Tafo experiments is a cross between two self-incompatible types from the Upper Amazon region and the Institute hopes to distribute seed of this cross from bi-clonal plots.

SUMMARY.

1. The insects responsible for much of the cross-pollination of cacao have been identified as :—*Forcipomyia quasi-ingrami* Macfie and *Lasiohelea nana* Macfie, in Trinidad ; *Forcipomyia ingrami* Carter, *F. ashantii* Ingram and Macfie, and *Lasiohelea litoraurea* Ingram and Macfie, in the Gold Coast.

2. The incidence of cross-pollination during 1946-1948 at Tafo, Gold Coast, was found to vary between 18 and 43 per cent. by the use of a recessive character, " albinoism ", to indicate self-pollinated progeny, and a dominant character, red pigmentation, to identify crossed progeny.

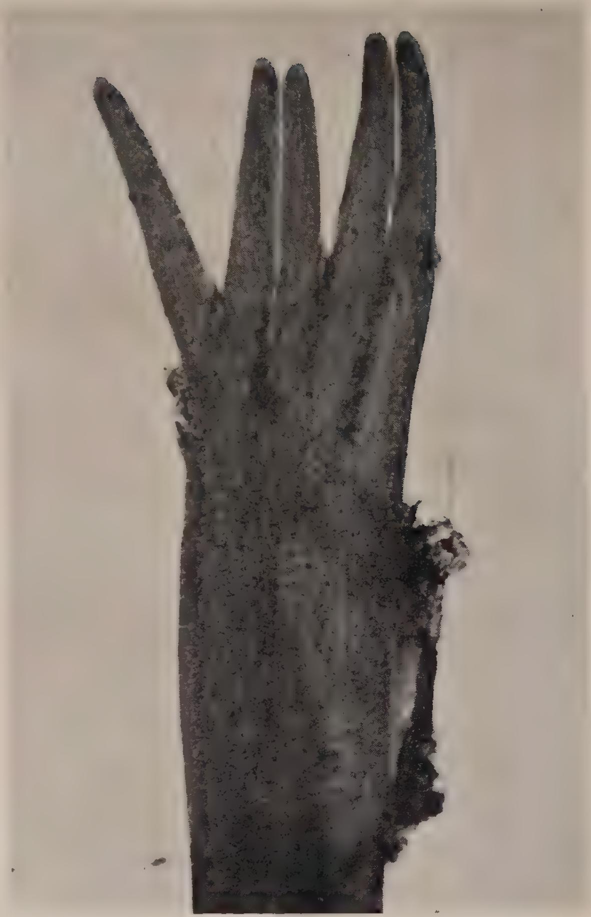

FIG. 1.

Photomicrograph of pistil of cacao flower which has been pollinated by a Ceratopogonid midge. Note on right side of the style the cluster of pollen grains pierced by a bristle (x 40).

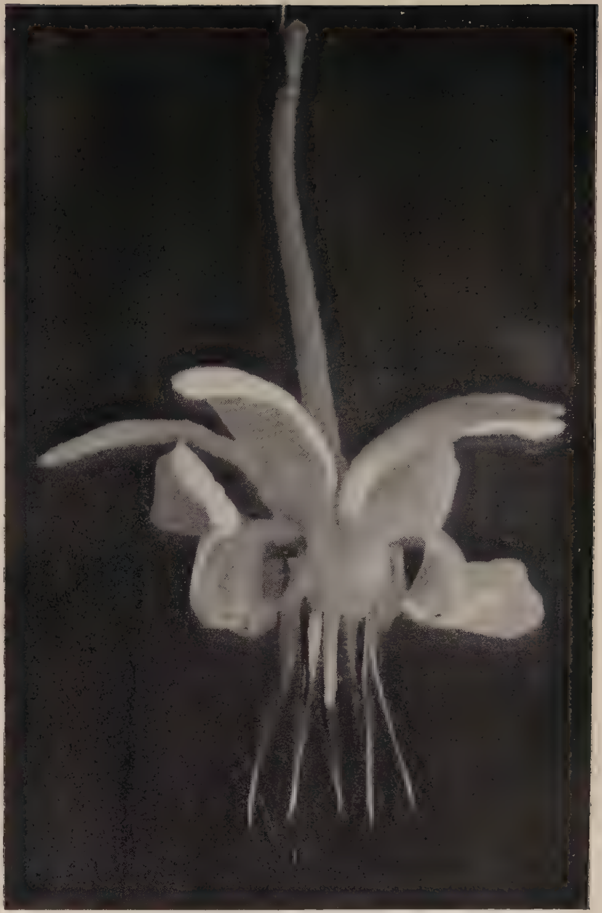

FIG. 2.

Cacao flower showing alignment of pistil and pigmented staminodes.

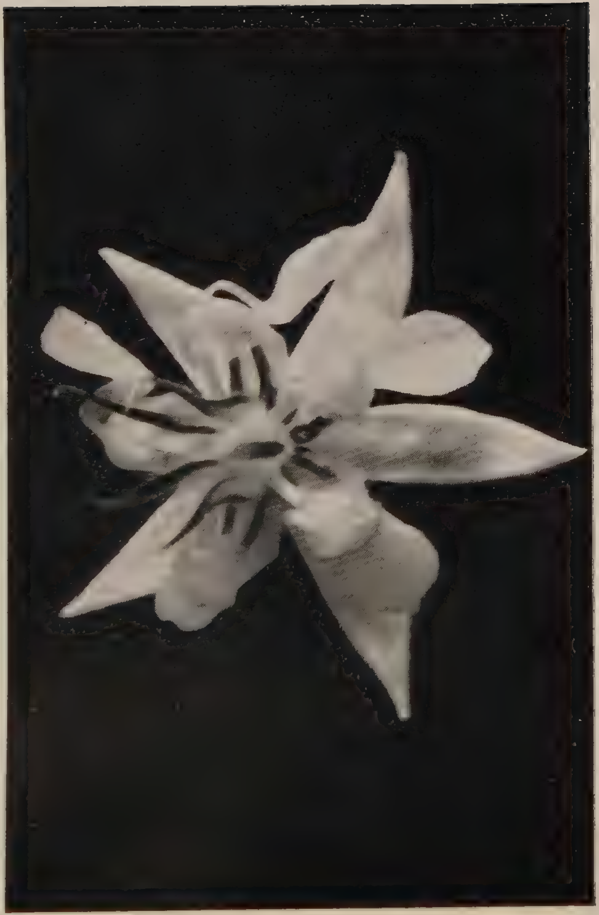

FIG. 3.

Cacao flower showing pigmented "guide-lines" inside the petal hoods which enclose the anthers.

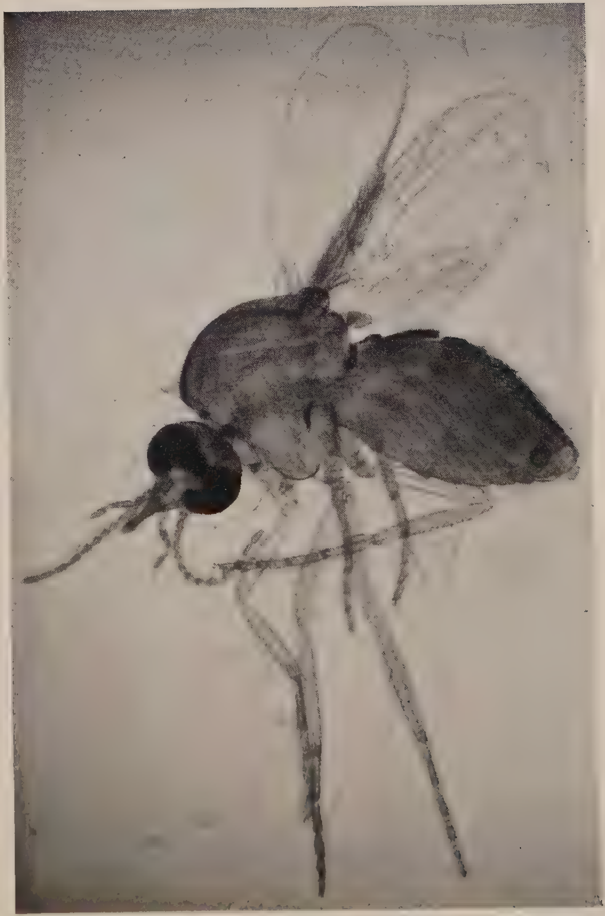

FIG. 4.

Forcipomyia ingrami : Carter. Note that many bristles have been detached in the mount. × 100 approximately.

3. The extent of cross-pollination was investigated and it was found that pollination occurred between trees two rows apart but less frequently than between adjacent trees.

4. Double pollination by different pollen parents was shown to occur. A second application of pollen effected some fertilization up to 6 hours after the first.

5. The design of clonal plots planted for seed distribution is discussed in regard to pollination factors.

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THE GROWTH FLUSHES OF CACAO

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In the periodic and discontinuous nature of its growth the cacao plant (*Theobroma cacao* L.) closely resembles the tea plant (*Camellia thea* Link.), the vegetative growth of which has been fully described by Bond (1942, 1945). Periods of active growth, with rapid elongation of the shoot and the development of new leaves, alternate with dormant periods. The process of growth is referred to as " flushing " and the young shoot and leaves as the " flush ".

The cacao plant is most susceptible to insect attack when flushing. Such major pests as the capsids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* Dist., cause their most severe damage at this time, while amongst minor pests aphids (*Toxoptera coffeae* Nietner) and psyllids (*Mesohomotoma tessmanii* Aulm) attack only the flush and flowers. Flushing is also of importance in connection with the two most serious diseases of cacao. The fungus of Witches' Broom disease in South America and the West Indies (*Marasmius perniciosus* Stahel) commonly invades the young flush (Baker and Crowdy, 1943) whilst the symptoms of swollen shoot virus of West Africa, which are first apparent in the leaves formed after infection, can be most readily detected in the flush (Posnette, 1947).

It is not surprising that attention has been directed to flushing in its relation to pests and diseases rather than to its effect on growth rate, and that this attention has been focused not on the growth of individual branches but on the spectacular " heavy " flushes which cover the entire canopy of the tree. It has, in fact, been argued that most of the major ills of cacao could be avoided if, instead of producing heavy flushes, the tree could be induced to spread the growth and renewal of foliage over a longer period, and recommendations for achieving this end by smoothing out fluctuations in environment were at one time made with confidence. However, the causes of flushing have received comparatively little scientific investigation and the " ideal " environment for cacao is now prescribed with less certainty.

MacDonald (1931, 1932) investigated the relationship between flushing and environmental changes in Trinidad and concluded that growth flushes coincide with changes in both atmospheric and soil moisture. Pound (1933) was unable to correlate flushing with environment and showed (1936) that the nutrient status of the soil had no effect on flushing. McKee (1944) found only slight differences in the flushing periodicity of six clones. Humphries (1944) suggested that the initiation of flushes was correlated with the maximum shade temperature and not with the other environmental factors he measured.

The observations here recorded were begun in the Gold Coast in 1938, at a time when it had been suggested that the rapid die-back of Gold Coast cacao was attributable to faulty environment (Dade, 1937). With the discovery in 1939 of swollen shoot virus they assumed secondary importance and experiments which the authors had hoped to make with seedling cacao at controlled temperatures and humidities were not carried out : from later observations it seems doubtful whether these experiments would have thrown much light on the behaviour of adult trees.

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The data presented support Humphries's conclusion that there is a critical temperature below which adult cacao flushes with difficulty ; they offer no explanation of the mechanism which leads to periodicity of flushing in seedling cacao or in adult trees maintained above the critical temperature. They include records of frequency and intensity of flushing collected from the same shaded and unshaded plots over a period of seven years and they are published mainly in the hope that these records may be of some help in subsequent investigations.

DAILY RECORDS ON INDIVIDUAL TREES.

During 1938-39 the degree of flush was recorded daily for 64 trees scattered at random through 60 acres of native-established cacao acquired by the Tafo Research Station. The trees were of uniform West African Amelonado type ; they were between 15 and 25 years of age, the only gross variables in their environment being shade and soil conditions.

INDEX OF FLUSH.

The following arbitrary standard was adopted :—

0 = no flushing.
1 = 1 to 6 shoots flushing.
2 = more than 6 shoots, less than half the canopy flushing.
3 = half to three-quarters of the canopy flushing.
4 = whole canopy flushing.

By using the mode of the daily readings as a weekly index, the 64 trees were distributed into weekly index classes. Surprising variation in the behaviour of the trees was found. There were only nine weeks in the year when less than half the trees were flushing ; during 23 weeks more than half the trees were flushing at degree 1 ; only once did more than 20 trees flush simultaneously at degree 2 ; 17 was the greatest number flushing together at degree 2, and 11 at degree 4.

Effect of shade.

A variable number of forest trees is left standing during peasant establishment of cacao. It was not difficult to select 12 trees unaffected by overhead shade and 12 others in sites where the number of shade trees was more than 6 per quarter-acre. On comparing the degrees of flush of the two sets of trees it was found that whilst at degrees 4, 3 and 2 there was no difference due to shade, there was a longer period at degree 1 on the unshaded plots and a longer period with no flush on the shaded plots, the differences being significant at the 2 per cent. point.

WEEKLY RECORDS OF FLUSHING ON SHADED AND UNSHADED PLOTS.

As a result of this indication that shade may affect the intensity of flushing, weekly recording was started in 1939 on three widely separated quarter-acre plots with no overhead shade and on three plots with fairly dense shade (chosen as near to the unshaded plots as possible). The same index of flush was used as in the previous records ; the weekly index given to each plot was that of the majority of trees in the plot. The mean number of cacao trees per plot was 112 : they were of the uniform Amelonado type and from 15 to 25 years of age.

Shade was provided by indigenous trees, most of which were deciduous. There was, however, always some shade over the cacao since each tree was without leaves for only a few weeks and different species, even individuals of the same species, were defoliated at different times. The shade trees were at heights ranging from about 5 to some 100 feet above the canopy of the cacao. The species represented, and the total number present on the three shaded plots were :—

> *Ricinodendron africanum* Muell. Arg. (10) ; *Celtis* spp. (4) ; *Triplochiton scleroxylon* K. Schum. (2) ; *Cola cordifolia* R. Br. (2) ; *C. acuminata* Schott & Engl. (1) ; *Sterculia rhinopetala* K. Schum. (1) ; *Cleistopholis patens* Benth. (1) ; *Pycnanthus kombo* Warb. (1) ; *Ophiobotrys zenkeri* Gilg. (1) ; *Terminalia superba* Engl. & Diels (1) ; *Conopharyngia cumminsii* Stapf (1) ; *Moreya spicata* Baill (1) ; *Funtumia elastica* Stapf (1) ; *Parkia bicolor* A. Chev. (1) ; *Erythrina altissima* A. Chev. (1) ; *Myrianthus arboreus* P. Beauv. (1) ; *Hannoa klaineana* Pierre & Engl. (1) ; *Treculia africana* Decn. (1) ; *Trichilia heudelotii* Planch. (1) ; *Ficus asperifolia* Miq. (1).

Measured by a photo-electric cell at noon, light intensity above the shaded cacao varied according to the degree of defoliation, but was usually about one-tenth of that above the unshaded trees.

The results were analysed after 45 weeks and, as shown in Table I, the unshaded trees flushed at a significantly greater intensity and for a longer period than the shaded trees.

TABLE I.

No. of plot weeks with different degrees of flush.

Flush Index.	0	1	2	3-4	Total plot weeks.
3 shaded plots	74	30	14	17	135
3 unshaded plots ..	22	34	39	40	135

chi-squared = 49·49, n = 3, sig. at 0·1.

These weekly observations were continued on the same three shaded and three unshaded plots until December, 1946, and, as shown in Table II, the difference between the flushing on shaded and unshaded plots was maintained.

TABLE II.

No. of plot weeks with different degrees of flush.

Flush Index.	0	1	2	3-4	Total plot weeks.
3 shaded plots	631	231	133	91	1,086
3 unshaded plots ..	366	257	229	234	1,086

chi-squared = 160·26, n = 3, sig. at 0·1.

In spite of soil differences, there was a great similarity in the incidence of flushing between the three shaded plots and also between the three unshaded plots. For more accurate comparison with the environmental data, flushing has been recorded in Figs. 1 and 2 only for the two plots, one shaded and one unshaded, on which the environmental records were taken.

It can be seen (Figs. 1 and 2) that the cacao tends to flush with a similar periodicity each year. There are usually two flushes in the dry season (December to March) a third early in the rainy season in May, a fourth during a period of comparatively high rainfall in October, and a fifth in November or early December at the end of the rainy season. The most consistent feature is the long dormant period from June to September, broken in only two years out of the seven. Flushing on the unshaded plots usually commences earlier and often reaches a greater intensity than on the shaded plots.

EFFECT OF SHADE ON ENVIRONMENT AND FLUSH.

In March, 1940, observations of environmental factors in one shaded and one unshaded plot were begun in an attempt to account for the effect of shade on flushing. Measurements were made of air temperatures and evaporating power, of soil moisture available in the surface foot, and later of sub-soil moisture to a depth of three feet and its relation to movements of the water-table.

Evaporation rate.

The evaporation rate was determined by white Livingston atmometers mounted on reservoirs of similar type and dimensions to those used by MacDonald (1931). One set of instruments was hung immediately above the cacao canopy, and another was placed mid-way between the trunk and the fringe of the canopy at a height of five feet above the ground ; readings were made twice weekly.

The super-canopy evaporation rate of the shaded plot was found to be slightly lower than the sub-canopy rate of the unshaded plot during the greater part of the year; it equalled or slightly exceeded it during dry periods when there was partial defoliation of the shade trees. Typical values for the four sets of instruments are given in Table III.

TABLE III.

Mean daily evaporation (mls.).

	Natural shade.		No shade.	
	Super-canopy.	Sub-canopy.	Super-canopy.	Sub-canopy.
3 wet months (July-Sept., 1941) ..	3·2	2·5	5·1	3·7
3 dry months (Jan.-March, 1942) ..	7·6	6·4	8·5	7·6

The sub-canopy readings for one year (1941) are recorded in Fig. 1 as mean daily rates over weekly periods. These values were found to be very closely correlated (r = + 0·959 for shaded plot : r = + 0·933 for unshaded plot) with weekly means for Minimum Relative Humidity measured by Edney hygrometers placed near the atmometers. It is clear from Fig. 1 that there is no correlation between changes in evaporation rate and the incidence of flushing in 1941 and this is equally true of other years.

Soil moisture.

Weekly determinations of soil moisture were made on samples taken from 0 to 6 in. and 6 to 12 in. at nine points on each plot. Available soil moistures are graphed in Fig. 1; they were obtained by subtracting the wilting coefficients (shade 0 to 6 in. = 3·9 per cent; 6 to 12 in. = 3·5 per cent. No shade 0 to 6 in. = 3·6 per cent.; 6 to 12 in. = 2·6 per cent.) from total moistures. The values are calculated for a depth of 0 to 12 in. and have been referred to the mid-point of the appropriate weekly period by taking the mean of two successive weekly determinations.

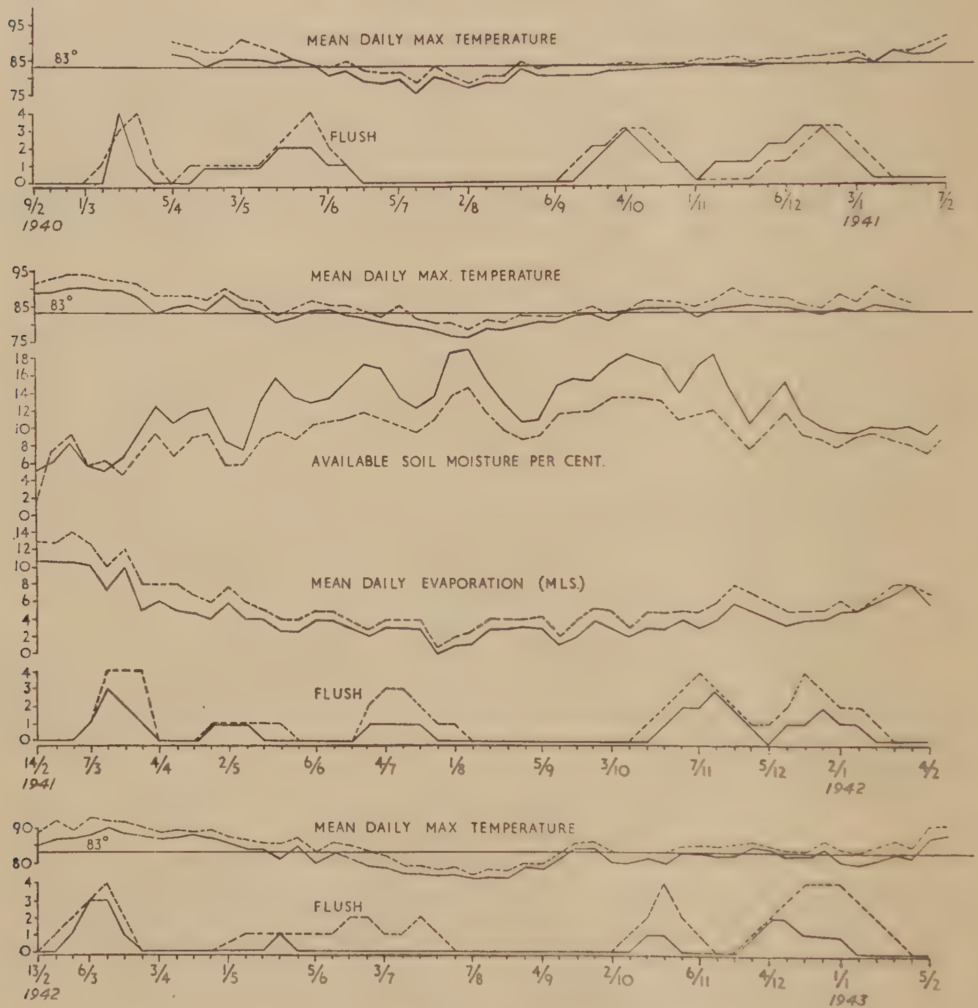

FIG. 1.

Flushing and environmental factors on shaded and unshaded plots 1940-1942.
Shaded plots, solid line. Unshaded plots, broken line.

Available moisture in the surface soils is closely correlated with rainfall. The values are consistently higher on the shaded plot, but the difference cannot be wholly attributed to the effect of shade for whilst the surface soils of both plots are sandy, the sub-soil of the shaded plot is much heavier than that of the non-shaded. This difference in sub-soil does not apply to the other pairs of plots on which flushing was recorded.

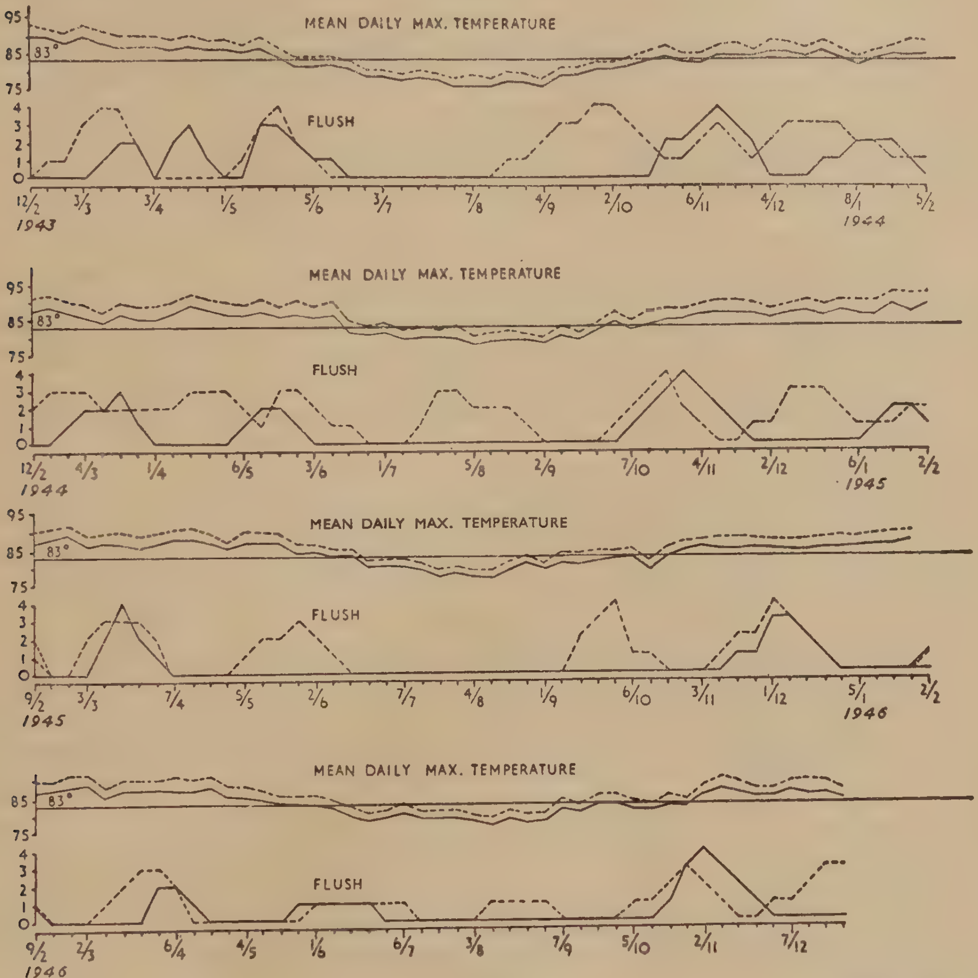

FIG. 2.

Flushing and weekly mean of daily maximum temperature on shaded and unshaded plots 1943-1946. Flushing lower graph. Temperature upper graph. Shaded plots, solid line. Unshaded plots, broken line.

The shaded plot has, therefore, a consistently lower evaporation rate and a higher content of available moisture in the surface soil. If either the onset or the maintenance of flushing is determined by the attainment or preservation of a critical balance between these two factors, a seasonal distinction might be expected between the incidence of growth flushes on the two plots. *None is apparent from our data.*

The only flush which consistently begins under conditions of rising soil moisture and falling evaporation rate is that which occurs in April or May, at the beginning of the rainy season and, in five years out of seven, flushing began earlier on the unshaded cacao rather than, as might be expected from the above hypothesis, on the shaded. A similar conclusion was reached by Pound, whose observations were made on cacao growing on sandy soils in which soil moisture was estimated from rainfall figures. MacDonald, on the other hand, collected his data from heavy soils (wilting coefficients 11 to 27 per cent.) and his moisture contents, measured from 0 to 12 in. are obviously affected by movement of the water table. Measurements of water-table and of sub-soil moisture to a depth of three feet were therefore made at Tafo from June, 1941, onwards, on the unshaded plot, and at points above and below the shaded plot (Greenwood, 1943). No connection could be found between the incidence of flushing and fluctuations in the water-table or the sub-soil moisture.

Sunshine.

Hours of sunshine and maximum temperature are closely correlated at Tafo and it would be difficult to differentiate the effects of the two factors. Attempts to measure radiation by means of differential readings of black and white atmometers were short-lived on account of the fragile nature of the black bulbs and the difficulty of replacement during the war. In Trinidad, where sunshine and temperature are less closely correlated, Humphries (1944) recorded " no obvious relationship between the duration of sunlight and flushing ".

Air temperatures.

Sub-canopy air temperatures were measured on the two plots by recording thermometers of bi-metallic strip type which were placed at the same height as the atmometers. They were screened vertically but not laterally, and readings were checked twice weekly by spot readings of maximum and minimum thermometers placed in the same screen.

Shade was found to cause a marked reduction in the diurnal temperature range. (Dry months, February to March : shaded = 16·7° F. ; non-shaded = 22·1° F. Wet months, July to August : shaded = 9·7° F. ; non-shaded = 11·3° F.) Minimum temperatures were from 1° to 3° F. higher and maximum temperatures 1° to 4° F. lower on the shaded plot ; the mean difference of maximum temperatures above 85° F. was 3·0°, and that below 85° was 2·0°.

Temperature-recording on the two plots ceased in April, 1943. In the three years during which the plot records had been taken, daily maximum and minimum temperatures had been measured in a Stevenson screen on an open site. The latter readings have been continued and from the previous parallel records the probable temperatures in the plots have been calculated to provide temperature records continuous with those for flushing from March, 1940, to December, 1946.

Humphries (loc. cit.) has suggested that 83° F. is a critical temperature for bud-bursting. The dates of bud-bursting were not recorded at Tafo ; they probably anticipated the dates for degree 1 flush by two weeks. It will be seen from Figs. 1 and 2 that there are many occasions when flushing begins within one or two weeks after the mean weekly maximum temperature has risen to about 83°. Good examples of this " trigger " effect are to be found in June, 1941 ; October, 1943 (shaded plot) ; July, 1944 (unshaded plot) ; October, 1944 (shaded plot) and September, 1945 (unshaded plot).

The only long dormant period occurs whilst the mean maximum temperature lies below 83° F. and the lower temperature of the shaded plots would account for their lag in flushing when this period is ended by the temperature rise usual in September. At temperatures over 83°F. flushes are separated by only short dormant phases and the lag of the shaded plots is less pronounced.

There are anomalies, some of which can be explained by reference to daily figures for maximum temperature. There is, for instance, a flush which began on the unshaded plot on 14th-21st August, 1943, when from the mean weekly figures it would appear that the maximum temperature had been below 83° F. for two months, whereas the maximum temperature recorded for 3rd August was 83° F. For others there is no ready explanation on a temperature basis : examples are the flushing of only the shaded plot in April, 1943, and of only the unshaded plot in June, 1945, when on both occasions the temperature on both plots was well above 83° F.

FLUSHING OF YOUNG CACAO.

Observations during the growth of young cacao seedlings in greenhouses and nurseries at Tafo had revealed no seasonal differences in flushing. At any period of the year, the first leaves after germination mature in about 25 days from planting the seed and the next three flushes follow at approximately monthly intervals. In order to determine at what age cacao seedlings cease to flush independently of environmental factors, the dates of flushing of two sets of uniform Amelonado seedlings were compared. One set of 40 seedlings was approximately six months and the other set twelve months old at the time the records were started. Both sets were kept under identical conditions of light and temperature, shade being provided by lath matting ; soil conditions were kept as uniform as possible by using the same potting soil and watering routine.

The records were continued for 18 months, from June, 1946, to November, 1947, during which time all but one of the 80 seedlings had flushed seven times, 76 seedlings had flushed eight times, and only 14 seedlings had flushed eleven times ; of these 14 seedlings, 13 were in the younger set. The number of seedlings flushing and the mean interval between the commencement dates for the first ten flushes are given in Table IV.

TABLE IV.

Mean intervals between flushes.

Flush.	Younger set.		Older set.	
	Number flushing.	Weeks since previous flush.	Number flushing.	Weeks since previous flush.
1	40	—	40	—
2	40	6·1	40	6·2
3	40	4·5	40	5·8
4	40	4·7	40	6·2
5	40	4·7	40	6·9
6	40	7·8	40	7·3
7	40	8·0	39	9·1
8	39	7·5	35	10·9
9	36	9·0	18	8·0
10	27	9·1	8	10·3

The interval between the first and second flushes seems to be disproportionately long and this may be due to slow recovery of some plants after potting. After the second flush the interval between flushes lengthens in both sets of seedlings, but the younger seedlings have significantly shorter dormant periods, or more frequent flushes, than the older seedlings ($t = 2{\cdot}4, n = 8$).

It is noteworthy that the younger seedlings, when they reached one year old did not have as short a dormant period as the older seedlings had had at that age. This fact, together with the abnormally low frequency of flushing at the end of the experiment, indicates the influence of a factor limiting growth. Observations on cacao seedlings growing in different sizes of pots, suggest that the factor is pot-binding restricting the development of new roots.

DISCUSSION.

It may appear that the effect of the cropping cycle on flushing has been ignored in this investigation. Observations strongly support the conclusion that although flushing may interfere with the growth of young fruit and contribute to the causes of physiological cherelle wilt, there is no reciprocal relationship. Young cacao of about four years old, not yet in bearing, has the same flushing cycle as mature cacao, and mature trees with and without a crop also flush together. The flushes which occur in November or December might be attributed to the removal of the main crop, but on the other hand there is seldom a flush following removal of the minor crop in June-July, and the September-October flush occurs when the trees are usually carrying their maximum number of pods.

A comparison of the flushing of mature cacao with that of young seedlings reveals a different reaction to environment, so that generalizations about bud-bursting of one will not necessarily apply to the other. This is particularly true of the interval between flushes, for which Humphries recorded one minimum of 4 weeks but concludes that 7 weeks is the usual minimum for mature cacao. With the single growing point on our seedlings, an interval of 3 weeks between the beginning of one flush and that of the next was the minimum, and was recorded on 12 occasions. An interval of 4 weeks was recorded 86 times in the younger set and 26 times in the older set of seedlings.

The conlusion which we draw from our data, supported by general observations is that the growing point of cacao has an " inherent " rhythm of maturation, differentiation and elongation (including further differentiation). The first two stages are the apparently " dormant " phase and the process of elongation is the flush. This rhythm can be repeated every 4 weeks ; a succession of five flushes at 4-weekly intervals has been recorded and a succession of three was frequently observed in seedlings less than one year old. There was a tendency for the dormant period to lengthen as the seedlings mature but intervals of six weeks were not uncommon on two-year-old seedlings and, up to this age, flushing seems to be independent of the temperature range at Tafo. Seedling flushes were recorded throughout the coolest months, and when mature cacao was not flushing.

The growth of mature cacao appears to be influenced by temperature, but we have not determined at what age or stage of growth this influence begins. Observations suggest, however, that after the formation of the second tier of branches (jorquette) flushing behaviour is synchronous with that of mature cacao, on which flushes can be expected to recur every 8 to 10 weeks while the daily maximum temperature under the cacao canopy remains above 83° F.

The possibility of shade trees directly influencing a crop by reducing temperature is less often considered than their effect on light intensity and on soil moisture and nutrient status. In relation to insect pests of cacao, the temperature effect of shade trees may exert a double influence, since higher temperatures may shorten the life history of the insect and also provide a greater food supply. While this appears to hold true for thrips, aphids and psyllids, which become much more prevalent during the periods of highest temperatures in West Africa, it does not seem to be true for capsids whose numbers decline at this time (Nicol, 1948).

The effect of temperature on the growth of cacao is seldom considered when new areas for cacao planting are discussed, although it would appear to be an important consideration. Temperature may well be the limiting factor in the growth of cacao at high altitudes ; the reason for the 2,000 ft. limit has never been explained satisfactorily. In countries where maximum shade temperatures are seldom above 83° F., it would seem desirable to grow cacao without shade, if other factors, such as thrips attack, permit.

Perhaps the most important fact emerging from these observations is that under Tafo conditions, which are typical of a large proportion of the cacao growing area in the Gold Coast, *most of the growth of mature cacao occurs in the drier months*, while during the main wet season months when conditions of water supply and humidity are most stable, little or no growth occurs. The need for further investigation of the optimum conditions for cacao cultivation can be stressed by quoting the view expressed by Cheesman (1948), who states that " the absence of a pronounced dry season may prove to be wholly favourable, as the cocoa tree flourishes best under even conditions of water supply and humidity and on the other hand reacts most unfavourably to violent fluctuations in these factors. Yet so little is known about the exact relationships between its flowering and cropping cycle and seasonal changes in the environment that only experiment can show whether cropping is increased or decreased by conditions approaching the optimum for vegetative growth. In other words, it may be necessary for maximum yield that the trees should experience some check to growth, such as is provided in most countries by a dry season."

SUMMARY.

1. Daily observations during 1938-39 on 64 mature cacao trees scattered among 60 acres of cacao at Tafo, Gold Coast, indicated that overhead shade influences the growth flushes of cacao.

2. Weekly observations on flushing were taken from 1939 to 1946 on three shaded and three unshaded plots. During part of this period records were taken of evaporation rate, soil moisture, sunshine and temperature on one shaded and one unshaded plot. Screen temperatures were recorded throughout the period.

3. It was found that unshaded mature cacao flushed more frequently and with greater intensity than shaded cacao, and that this difference was more marked during periods of low temperature.

4. The results indicate that the flushing of mature cacao is suppressed when the weekly mean of the daily maximum temperatures is below 83° F., and that no other environmental factors measured influence flushing.

5. Young cacao seedlings show regular periodic growth and, up to at least one year old, their flushing seems to be independent of temperature.

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IDENTIFICATION OF BLACK CURRANT VARIETIES

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In the field inspection of black currants the first necessity is to be able, while proceeding at walking pace, to pick out any plant that differs from its neighbours—whether on account of disease or because it is a different variety. The ability can be acquired at first only by studying different plants adjacent to each other—staring at them for an hour, if necessary—until the actual points of difference can be seen. When the cause of difference is varietal a further requirement is to be able to identify the stranger. The identification of black currant varieties is not easy, even after long experience has been gained but, given the necessary experience, it can be facilitated by careful use of a " key ".

The National Fruit Testing Station has in preparation a key embodying a complete description of all known varieties and this will be published in due course. The key given below refers only to the chief varieties and a few others, but has been drawn up for a particular purpose. The field inspection of black currants for reversion must be made during the second half of June (when the disease is most readily visible) and it is convenient to be able to recognize varieties at the same time. Many of the plants to be inspected are young and consist of growing shoots only. This key, therefore, includes only the characters of the growing shoots but they are sufficient to enable fruiting bushes to be identified.

In order to use the key successfully it is essential to bear in mind its limitations and the factors that cause some of the characters to vary. Notes are therefore included on the practical difficulties that may be experienced in the field. Recognition depends upon a combination of characters not all of which are invariably present at the same time. Mere absence of a character means little, and it is only when a character is both present and " typical " that it makes a positive contribution towards identification. Typical characters of the growing shoots are exhibited only under normal good growing conditions, and it is essential to see that such conditions are present before drawing any conclusions. Useful characters are details of colour and shape, and all are subject to variation. Unless these vagaries are appreciated, a key may lead to a wrong conclusion.

ORIGINAL VARIETIES.

In the first volume of the Journal of Pomology (1920) Hatton described the results of a study of black currant varieties by himself and J. Amos. They had collected more than 50 varieties, but found that only four were quite distinct—*Baldwin*, *Boskoop Giant*, *French* and *Goliath*. The remainder were indistinguishable from one or other of these, except in some small details at certain times of the year. Presumably these slight variations were due to the fact that these four varieties come nearly true from seed.

The position now is different, as many varieties introduced since 1920 are derived from crosses of pairs variously selected from the original four. These may sometimes show characters that are not obvious in any of the original varieties, but in general they exhibit characters from both their parents and it follows that identification of the newer varieties is made much easier by a thorough knowledge of the four originals.

Colour. Colour is the most striking of all characters, and is often visible at a considerable distance. It can be very misleading, as it is readily affected by the conditions ; nevertheless it is too valuable to be ignored for it usually gives the first indication that there are " rogues " in a crop, and often a clue to the variety. It should be used as an indication needing confirmation by other characters and not as sufficient in itself.

Each of the four original varieties has its own typical leaf colour and this is transmitted to some of its offspring. Although the difference, when typical, is readily seen in the field, it defies description on paper. Moreover all are rendered " atypical " by certain conditions such as lack of cultivation, tree shade, or heavy dressings of fertilizers. Except as a warning that strangers are present, leaf colour is best ignored by all but the fully experienced.

Each of the four varieties also exhibits characteristic colours on the stems and petioles in late June and these are extremely valuable, even to the novice, provided that it is certain that they are not abnormal, owing to the growing conditions.

The main part of the stem of *Baldwin* becomes pink about midsummer and the adjoining petioles are usually of the same colour, though occasionally they are green. The top few inches of the main stem and the adjoining petioles are green. This colouring is transmitted to some of Baldwin's offspring, though it may vary from pink to purplish pink, brownish pink, or almost bronze—possibly affected by the plant's other parent. All present-day black currants that have coloured stems in late June are either Baldwin, or derived from Baldwin as one parent. This colouring may be developed early by any condition that adversely affects growth, such as late planting, late pruning, drought, lack of cultivation or starvation. On the other hand it may be delayed if there is rapid growth, which may be caused by factors such as wet weather or nitrogenous manures, especially after an initial check.

The visible parts of the stems and petioles of *Goliath* are varying shades of light green, often pale. This character, in varying shades, is transmitted to some of its offspring. Some experience is needed to distinguish between these shades of green and those due to rapid growth. There is usually an inch or two of purplish colour at the base of the growth but it is not often readily visible. On only one occasion, in more than twenty years of observation, has this purplish colour been seen to extend upwards. In that instance about 1 per cent. of Cotswold Cross, growing on weedy uncultivated ground, showed this colour throughout the entire length of growing stems and petioles, but it could not have been mistaken for Baldwin pink.

Both *French* and *Boskoop* usually have green stems but the petioles develop some colour about midsummer, at the base, at both ends, or throughout the length. This contrast in colour is very striking, when it develops. Development of colour is affected by the same conditions as in Baldwin. In some seasons, however, the green stems may develop patches of pink, which are definite patches and therefore distinct from Baldwin pink, which is all round the stem. There is a difference between Boskoop and French, but it is not sufficient to form a distinguishing character. This matters little, because the leaves are very distinct and there are no known fruitful crosses bearing this character. The rogue known in England as I.G.P. Rogue and in

Scotland as Baldwin rogue, has a very distinct red mark at the base of the petiole (when it develops), a green stem, and leaves exhibiting characters like a French × Boskoop cross.

Leaf shape. Shape characters vary to a surprising degree and for reasons quite different from those affecting colour. Most of them are not affected at all by growing conditions. Details of shape differ from leaf to leaf on the same plant and it is even possible to select leaves from different varieties that are more nearly alike than leaves taken from a single plant of a single variety. It is, therefore, impossible to identify a variety by a single leaf, except occasionally by chance. What then is a typical leaf ? A few varieties produce an occasional leaf that is quite distinct from any produced by any other variety. Except for that, a typical leaf is the average of all the leaves on a plant, an average that cannot be ascertained by measurement but can only be estimated by eye. Therefore, the whole plant must be regarded at the same time, a distance of about three yards being necessary to obtain a correct view. Close inspection makes identification almost impossible and at first a definite effort to stand away may be needed. Details show up best in dull weather. If there is bright sun, it must be behind the observer and not in front.

The petiole of each of the four varieties holds the leaf in a characteristic manner, but this is not constant. That of *Baldwin* has a " swan " neck, holding the leaf vertically and pointing to one side (Fig. 1), though many of the leaves stand outright, especially when strong growth is crowded in a nursery row. That of *French* (Fig. 7) is not quite vertical and the leaf usually points directly downwards. The petiole and midrib of *Boskoop* (Fig. 2) often form a right angle. The leaf pose of *Goliath* (Fig. 9) is most distinct, as the petiole and midrib form a continuous curve, almost an arch, extending to the tip of the leaf, which is recurved.

Baldwin leaves appear of fine texture, while those of *Boskoop* are large and coarse, of *French* dark and leathery and of *Goliath* dark and very shiny. Baldwin and French leaves are relatively long and have acute serrations, but the leaf sinus of Baldwin varies from deep to medium and of French from medium to flat. Boskoop and Goliath have relatively short leaves, but there the resemblance ends. Boskoop sinus is usually very deep and the serrations are often crenate. Goliath serrations appear very numerous, small and almost rounded, while the leaf sinus varies around medium. Most of these points show up well in the photographs.

NEWER VARIETIES.

The various combinations of characters that can be used in identifying other varieties may now be detailed :—

(1) *Daniel's September* is a sport and difficult to distinguish from Baldwin. Typical differences are slightly sharper serrations, a flatter sinus, and occasional leaves showing variegation.

(2) There are four varieties having coloured stems indicating Baldwin, and more or less coarse veined leaves indicating Boskoop in their parentage. Of these *Wellington XXX* is nearest Baldwin. When not vigorous, the leaves are not very coarse, but at the end of June it can be distinguished from Baldwin by its brownish pink stem, absence of bloom, and shorter green growing tip. When very vigorous, the leaves are more like Boskoop but its coloured stem separates it from Boskoop at once. *Mendip Cross* leaves are dull and often appear greyish, especially at a

distance. They are shorter and coarser than those of Wellington XXX, i.e. they are nearer Boskoop. The stem colour is quite distinct, especially in late June when it becomes a deep purplish pink with bloom. The colour often runs half-way up the midrib in this variety and only occasionally does so in Wellington XXX. If any doubt is felt about stem colour another look in the first week in July will resolve it. After that, it disappears as the wood hardens. *Laxton's Giant* has a very dark, almost bronze-coloured stem. The leaves are coarse and have the Boskoop sinus but they are long, narrow and pointed. Occasionally a very distinct leaf is produced, pointed, unfolded and shaped like a gravy boat. *Laxton's Grape* has a leaf nearer Baldwin than Boskoop, but with a medium sinus.

(3) There are six varieties having coloured stems, indicating Baldwin, and leaves indicating both Baldwin and French. The best known of these—Westwick Choice and Blacksmith—are nearest to Baldwin; *Westwick Choice* leaf appears very long, but the surface is flat, sinus shallow and serrations deep. *Blacksmith* leaf is something like Baldwin, but has the flat surface of French. The serrations are very deep (deeper than in Westwick Choice) and all three lobes have long narrow points. The other four varieties—*Black Tony, Mite Free, Hatton Black* and *Supreme*—are not widely grown, are nearer French than Baldwin, and are difficult to distinguish from each other. All have leaves between Baldwin and French and all with shallow sinus. Those of Mite Free have very deep serrations, like Blacksmith; those of Hatton Black have small lateral lobes.

(4) There are two varieties having coloured stems, indicating Baldwin, and some other characters indicating Goliath. Neither is common. *Nigger* has long leaves, with deep sinus and very narrow at the stem end, where they are often cupped. Variegated leaves occur. *Malvern Cross* has its leaves crowded at the top, large, pointed, upfolded, with wavy edges and deep sinus. This variety shows the " double growths " (i.e. divisions of single shoots into two) which are typical of Goliath, as described by Hatton. The phenomenon is more frequent in Malvern Cross than in Goliath.

(5) There are three varieties with Goliath stems and with some Baldwin characters. *Cotswold Cross* leaf is dull green, short, with deep sinus and many small serrations, and is raised between the veins. *Davison's VIII* has a small, dull, slightly greyish leaf, with variable sinus. *Standard* has the growth habit of a red currant bush. It has a short, pointed greyish leaf, less rugose than that of Raven, with deep sinus.

(6) *Raven* has the Goliath stem and Boskoop habit of growth and, but for the stem, could best be described as a grey Boskoop. The grey-green, coarse leaves have very deep sinuses.

(7) There are two varieties with Goliath stems and with some French characters. *Westwick Triumph* is near Goliath, but many of the leaves are definitely " domed " (i.e.turn down all round). An occasional leaf has very small side lobes and appears almost round. The old leaves often show the dark leathery surface typical of French. *Matchless* has rather narrow, pointed Goliath-French leaves, with shallow sinus.

(8) The stem and petiole colours characteristic of Boskoop or French are not shown by any fruitful new variety but are exhibited by the " rogue " known in England as *I.G.P. Rogue*, and in Scotland as *Baldwin Rogue*. This plant should be known, as it is being sold under many ordinary names and it carries scarcely any crop. It is an exceptionally strong grower with very large leaves having Boskoop and

French characters and with old leaves sometimes having the dark leathery surface typical of French. About midsummer it develops deep red patches at the base of the petiole, so distinct as to be sufficient to identify it at that time. Every effort should be made to eliminate it.

KEY TO BLACK CURRANT VARIETIES IN LATE JUNE AND EARLY JULY

A. STEMS COLOURED : PETIOLES USUALLY COLOURED, OCCASIONALLY GREEN

Apparent derivation.	*Distinguishing characters.*	*Variety.*
Baldwin.	Leaves of fine texture, regular, acute, appearing long ; sinus usually deep.	BALDWIN.
Baldwin Sport.	Like Baldwin but sinus not so deep and serrations rather sharp. Occasional variegation. Some late fruit.	DANIEL'S SEPTEMBER.
Baldwin × Boskoop.	Habit of Boskoop. Leaves nearer Baldwin when growth weak but nearer Boskoop when growth strong. Stem brownish pink, no bloom, shiny.	WELLINGTON XXX.
Baldwin × Boskoop.	Habit near Baldwin. Purplish pink stem with bloom. Leaves short, coarse, soft, dull, greyish.	MENDIP CROSS.
Baldwin × Boskoop.	Dark stem, darker petioles. Long, narrow, pointed, coarse leaf, sometimes " dished " ; deep sinus.	LAXTON'S GIANT.
Baldwin × Boskoop.	Leaf nearer Baldwin than Boskoop ; rugose ; long terminal lobe ; sinus medium.	LAXTON'S GRAPE.
Baldwin × French.	Leaf appears very long, surface flat (not wrinkled), blade as a whole often upfolded ; sinus shallow ; serrations deep and acute.	WESTWICK CHOICE.
Baldwin × French.	Flat Baldwin leaf ; medium sinus ; all lobes very pointed ; serrations very deep and acute.	BLACKSMITH.
Baldwin × French.	Leaf nearer French than Baldwin.	FOUR LITTLE-KNOWN VARIETIES.
Baldwin × Goliath.	Many double growths. Leaves crowded at top of shoot ; large, long, pointed, upfolded, wavy edges, very rugose ; deep sinus.	MALVERN CROSS.
Baldwin × Goliath.	Long narrow leaves, small serrations. Often cupped at stem end.	NIGGER.

B. STEMS GREENISH : PETIOLES PALE

Apparent derivation.	*Distinguishing characters.*	*Variety.*
Goliath.	Leaves convex, very irregular, rather short, serrations small ; sinus medium. Old leaves dark and shiny.	GOLIATH.
Goliath × Baldwin.	Leaves green, smooth, dull, short ; sinus deep ; surface raised between veins ; many small acute serrations.	COTSWOLD CROSS.
Goliath × Baldwin.	Leaves acute, fine veined, dull ; sinus less deep, *not* pointed at stem end.	DAVISON'S VIII.

Apparent derivation.	*Distinguishing characters.*	*Variety.*
Goliath × Baldwin.	Short greyish leaf; deep sinus; not very rugose. Habit like that of a red currant bush.	STANDARD.
Goliath × Boskoop.	Grey-green coarse leaves; very deep sinus.	RAVEN.
Goliath × French.	Leaf of Goliath shape but domed; occasional rounded lobes. Old leaves leathery, shiny.	WESTWICK TRIUMPH.
Goliath × French.	Narrow pointed Goliath-French leaves, rugose, rather shallow sinus.	MATCHLESS.

C. STEMS GREEN : PETIOLES PINK OR WITH PINK MARKINGS

Apparent derivation.	*Distinguishing characters.*	*Variety.*
Boskoop.	Leaves large and coarse, lobes obtuse; sinus very deep, not pointed.	BOSKOOP.
French.	Young leaves not unlike Baldwin, but sinus shallow or flat. Old leaves flat, squarish, dark, leathery.	FRENCH.
Boskoop × French	Very strong upright growth. Bright red at base of petioles (when it develops). Very large long leaves. Old leaves dark and leathery. Reminiscent of Boskoop and French. Known in Scotland as Baldwin Rogue.	I. G. P. ROGUE.

NOTES ON KEY.

Since the above descriptions were written, East Malling Research Station has distributed the new late variety Amos Black, which is a cross between Baldwin and Goliath. This variety has not been known for long enough to say which are the most important characters for purposes of identification.

In the key the chief varieties of black currants are arranged in three sections according to the colours of their stems and petioles in late June and early July. The colours mentioned usually appear between 16th and 20th June and disappear again about 5th to 10th July.

The first section comprises those varieties that appear to take these colour characters from Baldwin; the second comprises those that in respect of colour, resemble Goliath; and the third contains Boskoop and French.

Within each section the varieties are arranged according to their apparent derivation and details are given for the separation of one from another. The characters given are the minimum considered necessary for identification.

A plant which does not fit any of the combinations of characters given may possibly be a worthless "rogue" originating as a chance seedling. Such rogues do occasionally occur.

ACKNOWLEDGMENTS.

Thanks are due to the National Fruit Testing Station for taking those photographs having a light background and to East Malling Research Station for those with a dark background. All the plates are reproduced by courtesy of the Ministry of Agriculture.

(*Received* 10/5/49.)

IDENTIFICATION OF BLACK CURRANT
VARIETIES. BY J. TURNBULL.

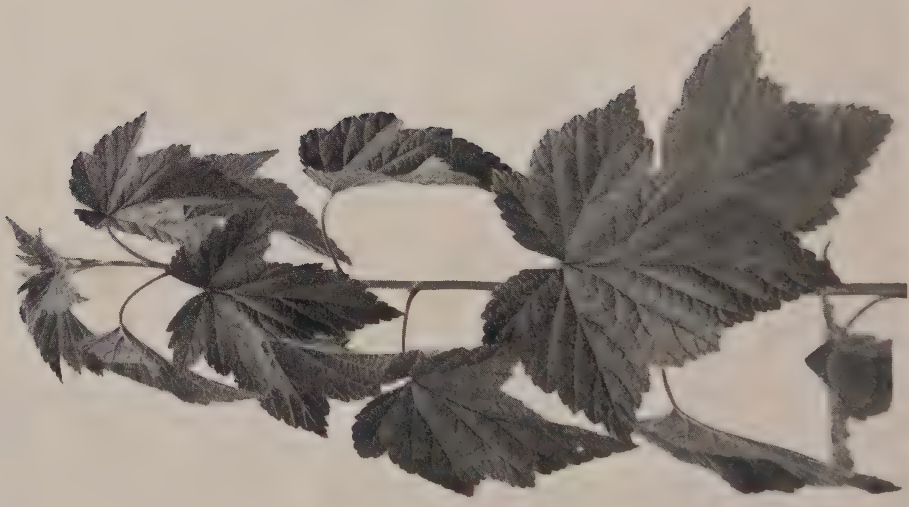

FIG. 1. Baldwin.

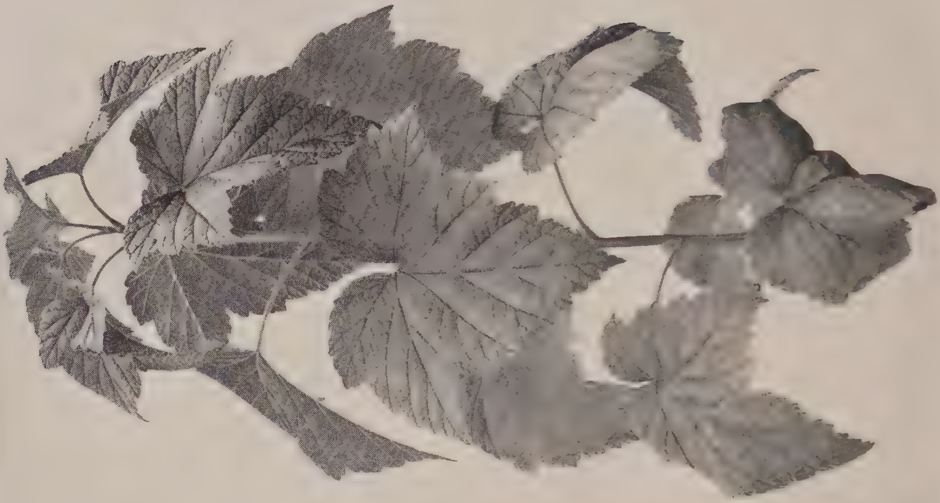

FIG. 2. Boskoop Giant.

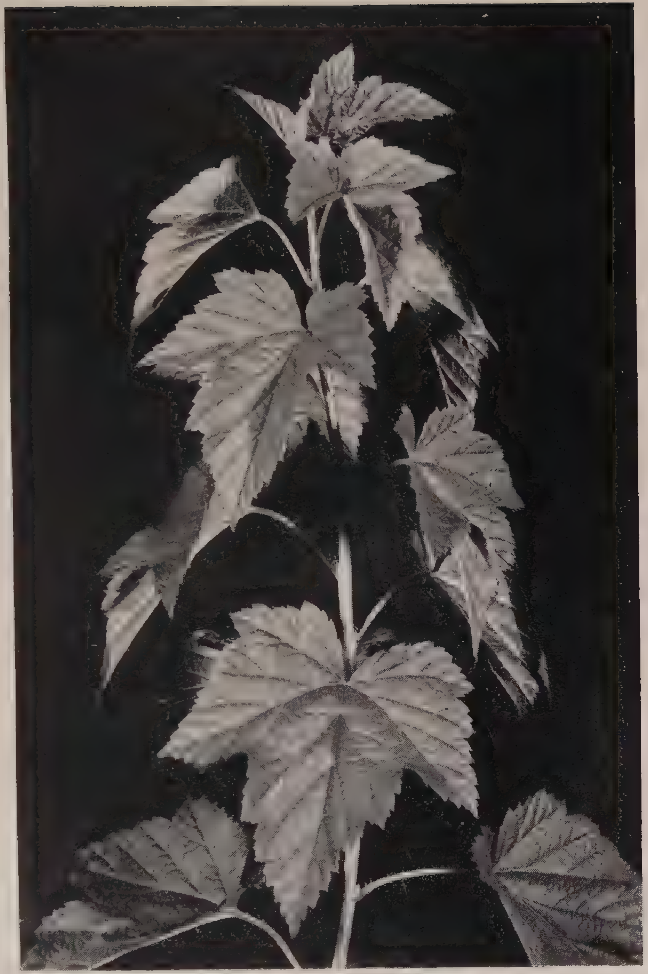

FIG. 3. Wellington XXX.

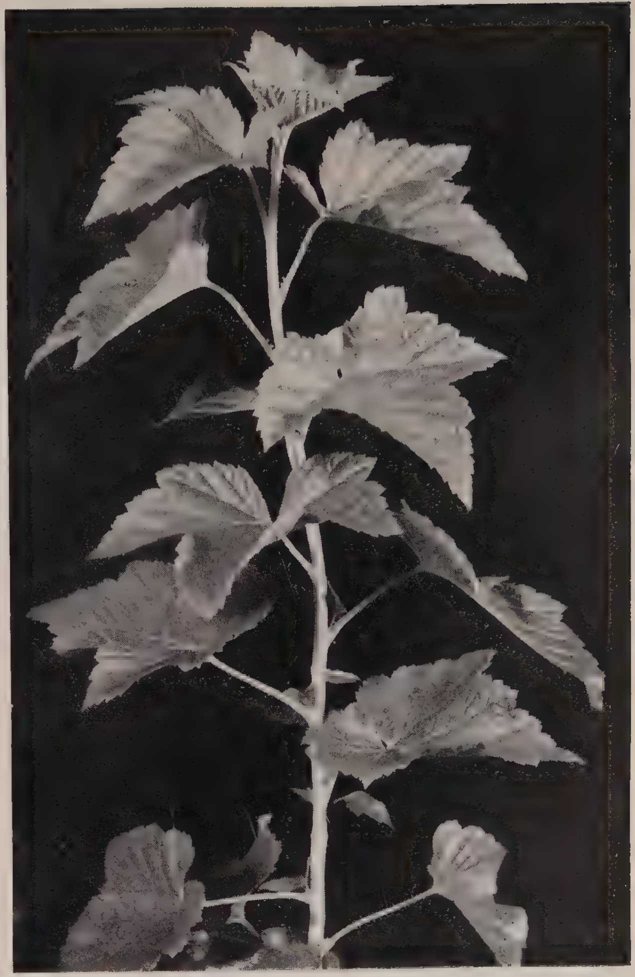

FIG. 4. Mendip Cross.

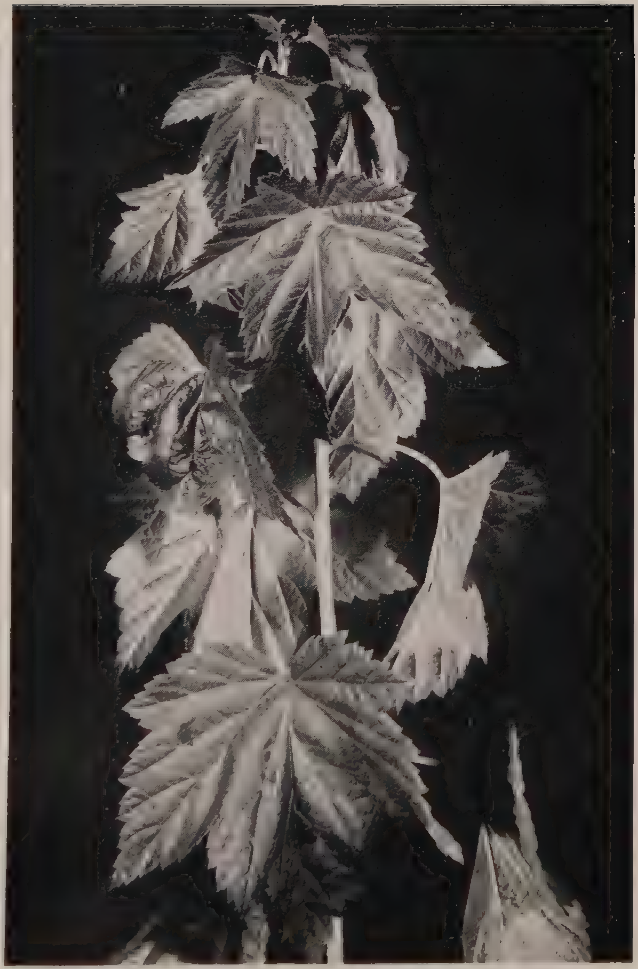

FIG. 5. Daniel's September.

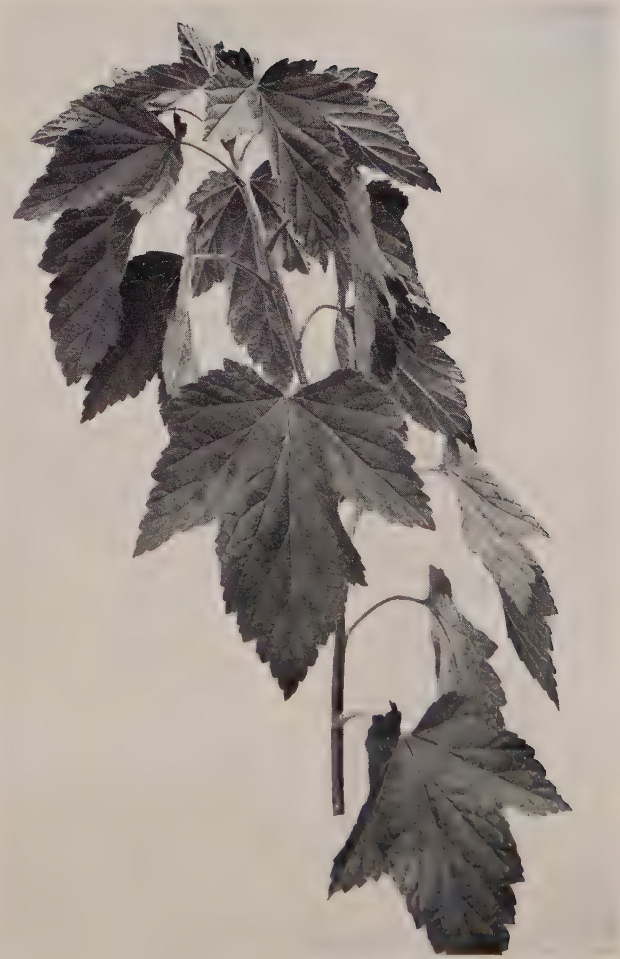

FIG. 6. Westwick Choice.

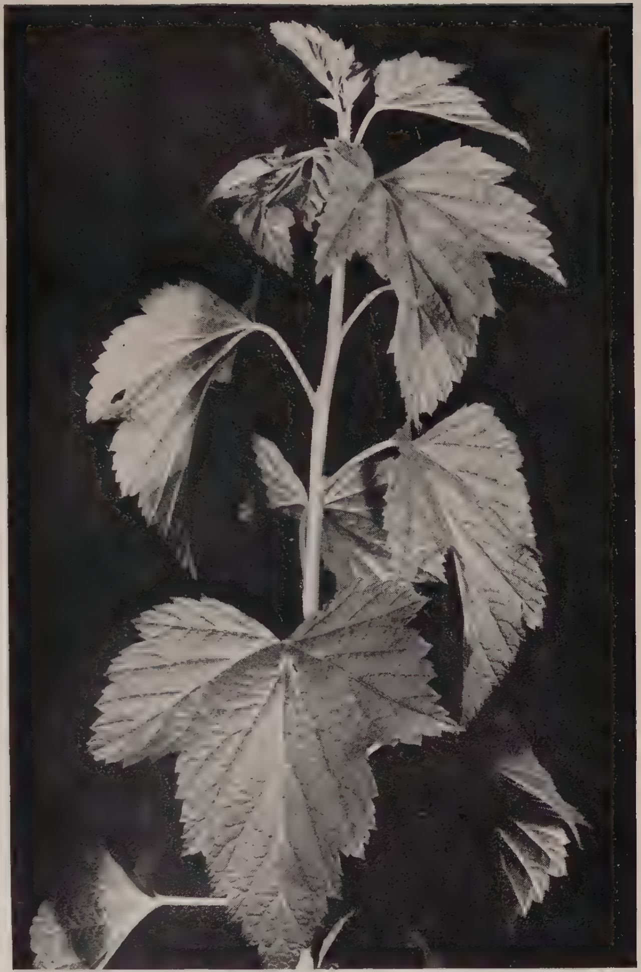

FIG. 7. French (Seabrook's)

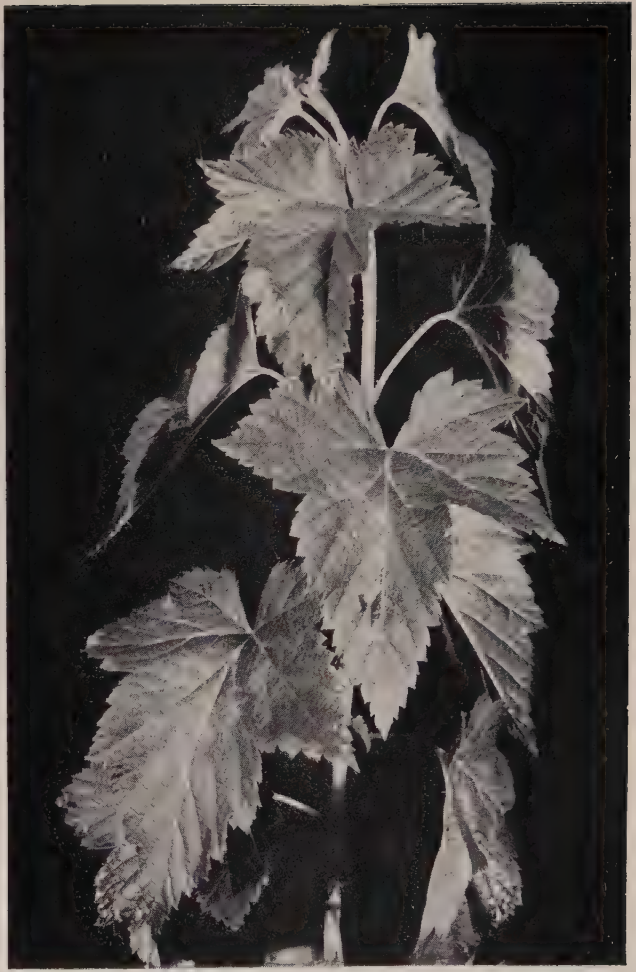

FIG. 8. Blacksmith.

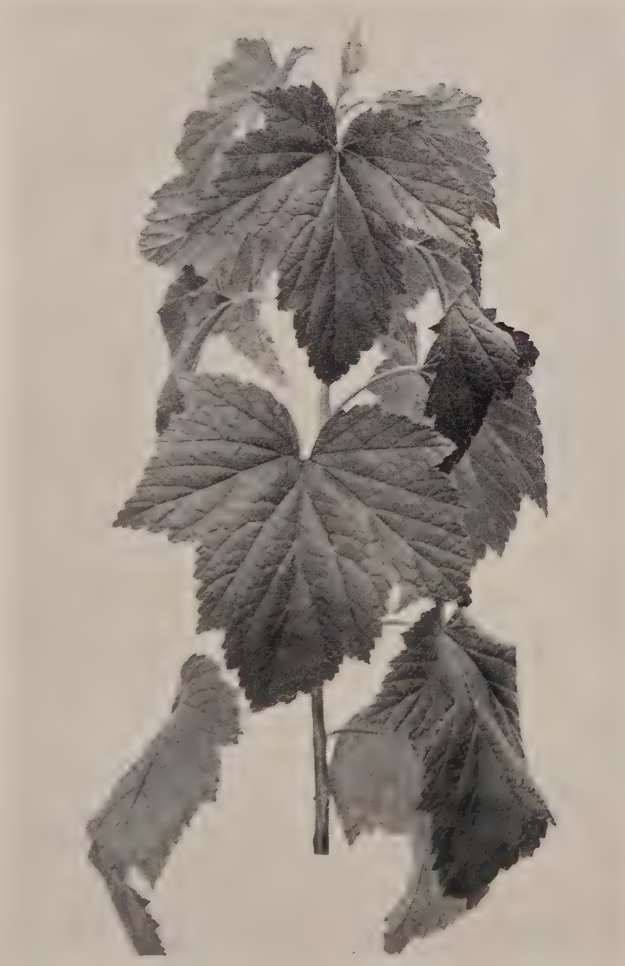

FIG. 9. Goliath.

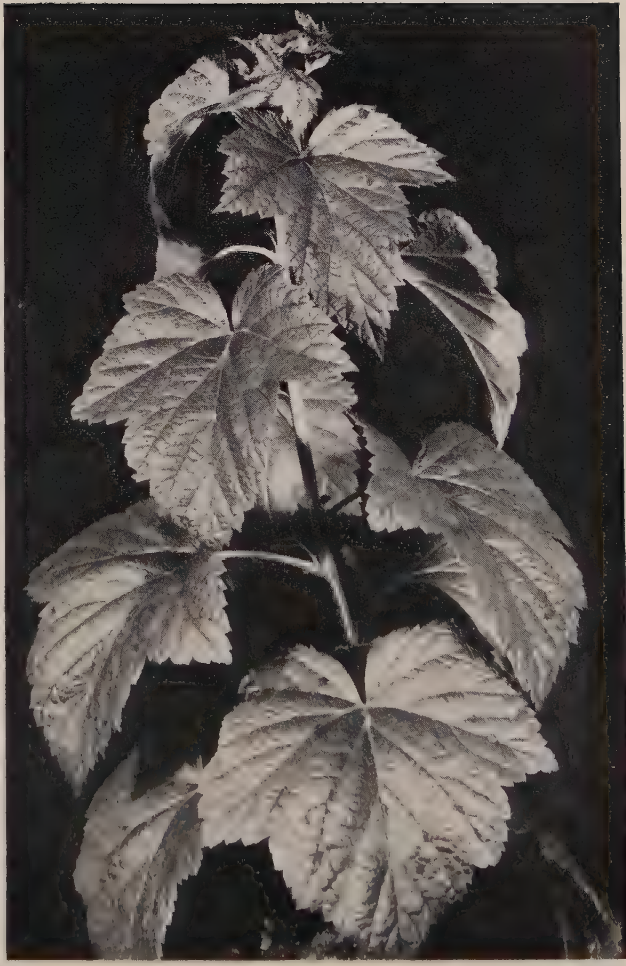

FIG. 10. Westwick Triumph.

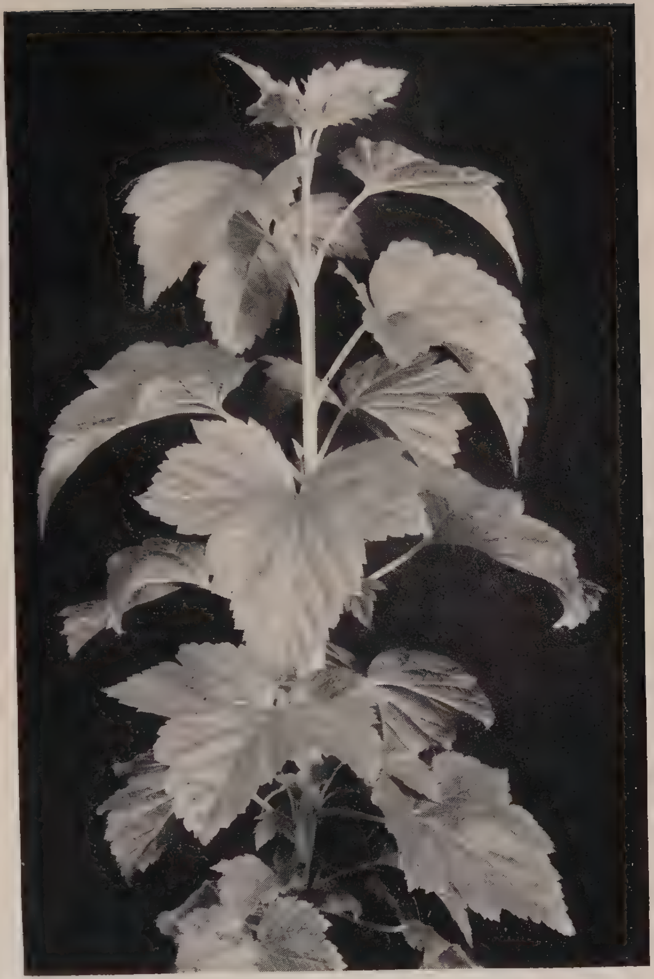

Fig. 11. Davison's VIII.

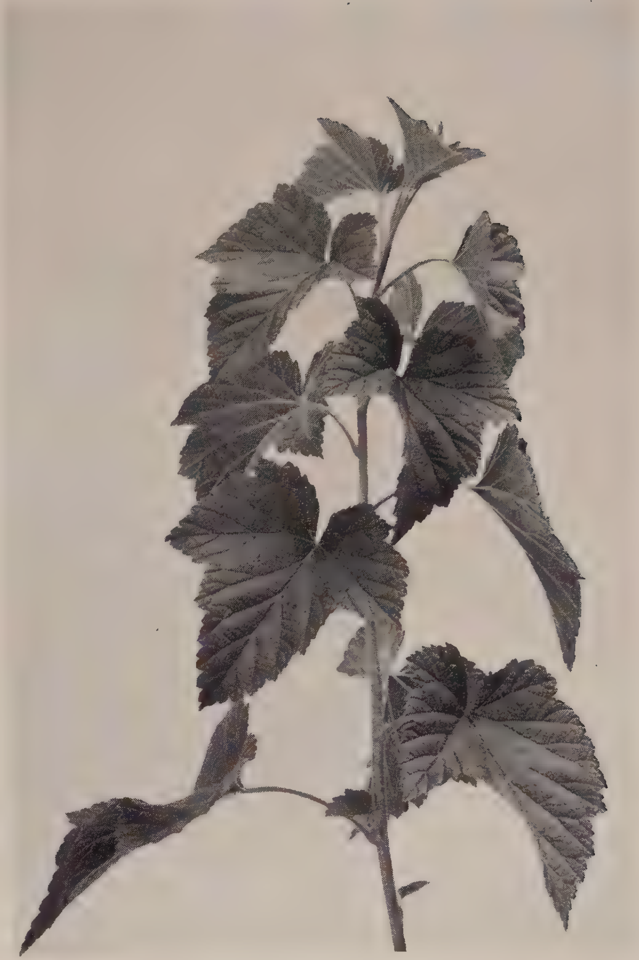

Fig. 12. Cotswold Cross.

RIPENING OF TOMATOES

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SYNOPSIS

Fully grown unripe tomatoes will ripen satisfactorily if kept in a warm, not too dry atmosphere. The rate of ripening of some fruits is, however, slow. Some tomato growers have sought to hasten ripening by using ethylene, a gas which is known to accelerate the ripening of many fruits, such as bananas or apples. Comparisons of the rates of ripening of tomatoes in atmospheres containing ethylene with others in atmospheres free from ethylene have shown that although this gas occasionally accelerates ripening, it usually has little or no effect.

INTRODUCTION.

IT is well known that tomatoes picked when fully grown but still green or with only faint signs of ripening, will usually ripen to reasonably good colour and flavour if kept in a warm room. The amateur grower of tomatoes usually strips outdoor plants at the end of the season and ripens the fruit indoors. Scientific literature contains references to artificial ripening of green tomatoes; thus Wright *et al.* (1931) report that tomatoes ripened normally from the green (with a white star at the stylar end of the fruit) or turning stages, when kept at 60°-70° F., and 85 per cent. relative humidity. Furlong (1946), of this laboratory, has also reported satisfactory ripening of green tomatoes at 65° F.

Often this artificial ripening takes several weeks to complete, and the fruits in any one sample do not all ripen at the same rate. The ripening of apples and some varieties of plum can be accelerated by ethylene, and this gas is occasionally used to even out the ripening of bananas. It is thus logical to enquire if the use of ethylene in the ripening room will either accelerate the rate of ripening of tomatoes or, by stimulating certain fruits, will even out the rates of ripening so that all the fruits in a sample are ripe at about the same time.

Furlong investigated this problem, using "Harbinger" tomatoes grown outdoors and picked on 31st October. His conclusions were that "ethylene at a concentration of 0·1 per cent. in the air accelerated the ripening of only the most slowly ripening fruits. The general effect was to make the ripening more uniform, but the rate of ripening of the sample as a whole was not accelerated to any great extent."

Since about 1946, several commercial growers have installed ripening rooms, in which unripe tomatoes can be kept at a high temperature in moist air, and treated with ethylene. Conflicting statements which we have heard regarding the efficacy of these rooms, and the quality of the fruit ripened in them, rendered further examination of the question desirable.

Many of the shipments of tomatoes from the Canary Islands, especially during the winter, arrive green. The scope of the investigations was, therefore, widened to cover these tomatoes also; some preliminary results are presented here.

EXPERIMENTAL.

A. *Description of fruit used.*

Three varieties of English tomatoes were used, namely "Stonor's M.P.", grown both outdoors and under glass, "First in the Field" and "Hundredfold", grown outdoors. Tables I to IV, below, give the dates on which artificial ripening began.

The fruit was picked the previous day, from a market garden at Swanley, Kent, and travelled up to London overnight by road.

The Canary Island tomatoes were obtained from commercial consignments to Covent Garden market.

Except where otherwise stated below, the English tomatoes were usually sorted into three different grades, viz. :

(*a*) " Green " : tomatoes with no sign of ripening. The fruits were either uniformly green, or showed a darker-green star at the calyx end. Some " green " fruits may not have been fully grown.

(*b*) " White " : tomatoes in this grade were definitely fully grown and the uniform green colour had begun to break at the stylar end, producing a light-green, almost white, star.

(*c*) " Turning " : showing either patches of pink colour, or with a general yellowing of the ground colour.

Canary Island tomatoes were sorted into two grades, viz. : " green " and " turning ". Many of the green fruits appeared to be immature.

As ripening proceeded, the fruits were classed as " ripe " at a stage which lay between the grower's and the consumer's opinion of ripeness. Thus the " ripe " fruits were uniformly coloured a vivid scarlet, and the seeds were no longer green. The tomatoes were still very firm, and the full flavour had not developed.

B. Methods.

After grading as above* the tomatoes were sorted into samples of 50 fruits each and stored on grease-proof paper on wooden trays. One or more trays were used to study the effect of a given set of conditions on ripening. The trays were stored both at constant temperature (65° F. or 70° F. ; 18·3° C. or 21·1° C.) in air or in air containing ethylene, at a relative humidity of 85 to 90 per cent., and in air at fluctuating temperature, similar to that prevailing in market warehouses. Full details of the treatments used are given in Tables I to IV.

The treatment with ethylene was given in gas-tight tanks, placed in the constant-temperature chambers. The tanks were constantly ventilated at rates sufficient to prevent accumulation of carbon dioxide, ethylene being added to the air stream at a concentration of 0·1 per cent. When bananas or other fruits are ripened by ethylene, it is not necessary to continue treatment with the gas until the fruit is ripe ; treatment for about 2 days is usually sufficient. For that reason, treatment of the tomatoes with ethylene was not kept going all the time, but for periods of two to twelve days at the beginning of the experiment, followed by air alone.

The tomatoes from the Canary Islands were treated either in a similar fashion, or were ripened in the boxes without unpacking.

Examinations were made each day or each two days, and records kept of the number of " turning " and " ripe " fruits, as well as the percentage of rotted fruits. The percentages of ripe fruits were plotted against time and, from the smoothed curve, estimates were made of the number of days taken for 25 per cent., 50 per cent., 75 per cent., and 100 per cent. of the sample to ripen.

The experiments were terminated after 18 to 21 days, since any artificial ripening process which takes three weeks to complete, on a constantly-cropping fruit, is not likely to have commercial possibilities except in the final clearing of green fruit from an outdoor crop.

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* Except fruit received on 27th August, 1948, which was not graded.

C. *Results.*

Canary Island tomatoes differed from the English in two respects. They were not put under controlled conditions until about two weeks after picking (and had been exposed to relatively low temperatures during part of that time), and secondly many of them appeared to be not fully grown. For these reasons, the results for the two types of fruit are presented and discussed separately.

(*a*) *English tomatoes.* The speeds of ripening of the different samples are summarized in Tables I to IV, which also give details of the experimental conditions. In some samples of initially "green" or "white" fruits, all the unripe tomatoes reached the "turning" stage some days before ripening of the sample as a whole was complete. This has been shown in the tables (see e.g. Footnote ** to Table I).

TABLE I.

Ripening of "Stonor's M.P." tomatoes. Grown in glasshouse. (Observations on single samples of 50 fruits.)

Date experiment began.	Conditions of experiment.	Initial condition of fruit.*	Days taken to reach following percentages of ripe fruit.			
			25%	50%	75%	100%
27.8.48.	Air. 70°F.†	Unsorted, all grades from green to turning	5	8½(T)**	10	18
	Air. 67°F.‡		5	8 (T)	11	18
	Air + 0·1% ethylene, 2 days.§ 70°F.		5	8 (T)	10	13
	Air + 0·1% ethylene, 6 days.§ 70°F.		3½	5 (T)	6	10
14.9.48.	Air. 65°F.	Green.	7	9½	11 (T)	21
	Air. 62°F.‖	"	8	10½(T)	13	17
	Air + 0·1% ethylene, 6 days.§ 65°F.	"	6½	7½	8½(T)	11
14.9.48.	Air. 65°F.	Turning.	3½	4½	5½	10
	Air + 0·1% ethylene, 6 days.§ 65°F.	"	6	7	7	8
14.9.48.	Air. 65°F.	White.	6	7½	9½(T)	14
	Air. 62°F.‖	"	5	6	10½(T)	19
	Air + 0·1% ethylene, 6 days.§ 65°F.	"	6(T)	7	8	10
	Air + 0·1% ethylene, 12 days.§ 65°F.	"	6(T)	–	–	12

No rotting occurred in any of the samples.

* "Green"—definitely not beginning to ripen;
"White"—doubtful if beginning to ripen (see text),
"Turning"—with signs of pink or red colour.
† Constant temperature.
‡ "Market temperature" store, mean 67°F., max. 73°F., min. 59°F.
§ In air alone thereafter.
‖ "Market temperature" store, mean 62°F., max. 68°F., min. 56°F.
** (T) indicates that rest of sample was "turning".

TABLE II.

Ripening of "Stonor's M.P." tomatoes. Grown outdoors. (50 fruits/sample.)

Date experiment began.	Conditions of experiment.	Initial state of fruit.*	No. of samples.	Mean number of days to following percentages of ripe fruit.				Rotting.
				25%	50%	75%	100%	
14.9.48.	Air. 65°F.†	Turning	3	12	18	>21	–	36%, day 21.
	Air. 62°F.‡	,,	2	14	>21	–	–	11%, ,, ,,
	Air + 0·1% ethylene, 6 days.§ 65°F.	,,	2	8½	11½	20	>21	35%, ,, ,,
	Air + 0·1% ethylene, 12 days.§ 65°F.	,,	3	–	13	>21	–	45%, ,, ,,
14.9.48.	Air + 0·1% ethylene, 6 days.§ 65°F.	Green.	1	12	20(T)‖	–	–	Nil, day 21.
12.10.48.	Air. 75°F.†	Green.	1	11½	13½	15	>18	4%, day 18.
	Air. 65°F.†	,,	1	14½	18	–	–	10%, ,, ,,
12.10.48.	Air. 75°F.	Turning.	3	9	11	14½	>18	5%, day 18.
	Air. 65°F.	,,	3	13	18	–	–	12%, ,, ,,
	Air + 0·1% ethylene, 75°F. 2 days.§	,,	1	8	10½	14½	>18	8%, ,, ,,
	Air + 0·1% ethylene, 75°F. 4 days.§	,,	1	8½	12	15	>18	4%, ,, ,,

* As Table I.
† Constant temperature.
‡ As Table I.
§ In air thereafter.
‖ (T) indicates rest of sample was "turning".

Before considering the significance of the results given in Tables I to IV, it is necessary to know how reliable the estimates are. There was no experiment in which there was a large enough number of comparable replicates to yield a stringent statistical evaluation of error. It is, nevertheless, possible to get some idea of the magnitude of the errors. There were five treatments each in triplicate and four in duplicate. In these samples the time taken for the slowest ripening sample to reach a given stage varied from 0 per cent. (i.e. no difference) to 80 per cent. longer than the fastest time. The average difference was 20 per cent. Thus, it is not unreasonable to ignore any difference between the time of ripening of two samples, which does not exceed 20 per cent. The data are conflicting and difficult to interpret. Whether one considers the comparative effect of ethylene on a given variety of tomato, either at different times in the season or at different stages of maturity, or on different varieties of tomatoes picked at the same time or same stage of maturity, no logical picture emerges.

There were several experiments in which ethylene appeared to hasten the ripening of the fruit, gains being registered at each of the arbitrarily chosen points given in the tables, two experiments in which no effect was noticeable until after 75 per cent. of the fruit was ripe, and others where ethylene had no effect at all.

TABLE III.

Ripening of outdoor grown " First in the Field " tomatoes. (50 fruits/sample.)

Date experiment began.	Conditions of experiment.	Initial state of fruit.*	No. of samples.	Mean number of days to following percentages of ripe fruit.				Rotting.
				25%	50%	75%	100%	
27.8.48	Air. 70°F.†	Unsorted	1	11(T)**	13	15	>19	5%, day 19.
	Air. 67°F.‡	,,	1	12(T)	15	17	>19	9%, ,, ,,
	Air + 0·1% ethylene, 2 days.§ 70°F.	,,	1	11	13	16 (T)	>19	6%, ,, ,,
	Air + 0·1% ethylene, 6 days.§ 70°F.	,,	1	10	11	13 (T)	18	2%, ,, ,,
14.9.48	Air. 65°F.†	Green	1	17½	19	>21	–	8%, day 20.
	Air. 62°F.‖	,,	1	>21	–	–	–	Nil.
	Air + 0·1% ethylene, 6 days.§ 65°F.	,,	1	11½	16	20	>21	6%, ,, ,,
14.9.48	Air. 65°F.	White	1	15	18½	20½	>21	4%, day 20.
	Air. 62°F.	,,	1	21	–	–	–	Nil.
	Air + 0·1% ethylene, 6 days. 65°F.	,,	1	14	16½	19½	>21	8%, ,, ,,
12.10.48	Air. 75°F.†	Turning	3	9½	13	15	>18	19%, day 18.
	Air. 65°F.†	,,	2	12½	c. 19	–	–	34%, ,, ,,
	Air. 56°F.‡	,,	1	c.19-20	–	–	–	2%, ,, ,,
	Air + 0·1% ethylene, 4 days.§ 75°F.	,,	1	8	11	14	18	Nil.
	Air + 0·1% ethylene, 6 days.§ 75°F.	,,	1	9½	12	14	>18	4%, ,, ,,

*, †, ‡, §, **—As Table I.

‖ " Market temperature " store, mean—62°F., max.—68°F., min.—56°F.

†† " Market temperature " store, mean—56°F., max.—62°F., min.—48°F.

TABLE IV.

Ripening of outdoor grown " Hundredfold " tomatoes. (50 fruits/sample.)

Date experiment began.	Conditions of experiment.	Initial state of fruit.*	No. of samples.	Mean number of days to following percentages of ripe fruit.				Rotting.
				25%	50%	75%	100%	
27.8.48	Air. 70°F.†	Unsorted	2	11	14½(T)**	16½	19	6%, day 19.
	Air. 67°F.‡	,,	1	16	18	>19	—	7%, ,, ,,
	Air + 0·1% ethylene, 2 days.§ 70°F.	,,	1	9	12(T)	14	19	2%, ,, ,,
	Air + 0·1% ethylene, 6 days.§ 70°F.	,,	1	9(T)	11	12	13	8%, ,, ,,

*, †, ‡, §, **—As Table I.

In detail, these results are as follows :

(i) Experiments in which ethylene appeared to accelerate the ripening of all the fruits in the sample :

" Stonor's M.P." variety, glasshouse, picked 27.8.48, ungraded, (Table I) : the samples kept in air + 0·1 per cent. ethylene for 6 days at 70° F. ripened in about 60 per cent. of the time taken by the sample in air. This acceleration was noted at the 25, 50, 75, and 100 " percentage ripe " points.

" Stonor's M.P.", glasshouse, picked 14.9.48 at the " white " stage (Table I) : if the criterion that differences must exceed 20 per cent. is applied to the data, there is no evidence that ethylene has markedly accelerated the rate of ripening, but it will be noted that the samples treated with ethylene all reached the turning stage earlier than those in air (6 days as against 10), and ultimately the two samples treated with ethylene were ripe about 2 to 4 days before the air sample.

" Stonor's M.P.", outdoor, picked 14.9.48, turning, (Table II) : the tomatoes in this experiment rotted badly and this complicated the picture. However, it can be seen from Table II that whereas the sample in air at 65° F. took 12 and 18 days to reach the 25 per cent. and 50 per cent. ripe stages, those treated with ethylene did so in about 70 per cent. of the time.

" First in the Field ", outdoor, picked 14.9.48, initially green, (Table III) : again, the results are not clear-cut, because of the very slow rate of ripening at 65° F., but 25 per cent. of the fruits treated with ethylene were ripe in 11½ days, or six days less than the time taken by the sample in air. It is probable that this effect would have persisted to the 100 per cent. ripe stage, had the experiment been continued long enough.

" Hundredfold ", outdoor, picked 27.8.48, ungraded, (Table IV) : treatment with 0·1 per cent. ethylene for 6 days resulted in acceleration of ripening of all the fruits (note the 40 per cent. saving in time for complete ripening, and earlier " turning ").

(ii) Experiments in which ethylene accelerated the ripening of a fraction of the sample only :

" Stonor's M.P.", glasshouse, picked 27.8.48, unsorted, given ethylene for 2 days only, and picked 14.9.48, green, given ethylene for 6 days, (Table I) : these experiments gave the same result as Furlong (*loc. cit.*) had noted, namely that there was no significant acceleration of ripening until after 75 per cent. of the sample was ripe, but there was a marked reduction in the time taken for the remaining 25 per cent. to ripen.

(iii) Experiments in which there was no effect :

Ethylene had no effect on the rate of ripening in the following experiments :

" Stonor's M.P. ", glasshouse, picked 14.9.48, turning, 6 days ethylene, (Table I).

" Stonor's M.P.", outdoor, picked 12.10.48, turning, 2 and 4 days ethylene, (Table II).

" First in the Field ", outdoor, picked 27.8.48, unsorted, 2 and 6 days ethylene, (Table III). In this experiment the sample in air " turned " before those in ethylene.

"First in the Field", outdoor, picked 14.9.48, "white" stage, 6 days ethylene, (Table III).

"First in the Field", outdoor, picked 12.10.48, "turning" stage, 4 and 6 days ethylene, (Table III).

"Hundredfold", outdoor, picked 27.8.48, unsorted, 2 days ethylene, (Table IV).

Before presenting the results obtained with Canary Island fruit, there are certain observations of general interest to record.

The rate of ripening at "market temperature" was not always slower than at the higher constant temperature. Thus (Table I), "Stonor's M.P." tomatoes picked "white" on 14.9.48 ripened more slowly at 62° F. than at 65° F., but the green fruits ripened more quickly at the lower temperature. This is presumably an indication that errors of sampling may have been greater than the 20 per cent. postulated above.

Our grading into the three grades of maturity, "green", "white" and "turning" was on the whole satisfactory. Thus (Table I) "Stonor's M.P." tomatoes picked on 14.9.48 and graded into these three grades ripened in 17, 14 and 10 days respectively.

The flavour of the artificially ripened tomatoes was good and, so far as could be judged, compared favourably with that of ripe fruits picked at the same time. Shrivelling of the originally green fruits was occasionally observed, however. Some of the fruit which took a long time to ripen had a rather blotchy appearance but on the whole no abnormalities in ripening were observed.

(*b*) *Canary Island tomatoes.* Mid-season fruit, picked from 21st to 24th March, 1948, and received at the laboratory on 6th April, was used for the first experiments. Some of the boxes were put in the ripening rooms without being unpacked (except for an examination of the top layers of fruit to get an estimate of maturity), and some were unpacked and the fruit was stored on trays.

Table V summarizes the progress of ripening of the fruit on trays, and Table VI analyses the data as they apply to fruits which were initially either green or turning.

TABLE V.

Ripening of Canary Island tomatoes (on trays).

Days.	Percentage of Ripe Fruit.		
	At 45°F. in air.	At 66°F. in air.	At 66°F. in air + 0·2% ethylene.*
Mark A.§ *2 to 2¼" diameter.*			
At the start	27	27	27
3	—	63	66
6	42	92	85
Mark B.§ 1½" diameter.			
At the start	24	24	24
3	—	50	60
6	36	71	84

* Three days only, afterwards in air.

§ i.e. The grower's mark.

TABLE VI.

Ripening at 66° F. of initially green and "turning" Canary Island tomatoes. (Further analysis of data of Table V.)

Days.	Percentage of ripe fruit.			
	Originally green.		Originally turning.	
	Air.	Air + ethylene.	Air.	Air + ethylene.
Mark A.§				
3	5	9	95	100
6	95	59	100	100
Mark B.§				
3	Nil	16	91	100
6	96	72	100	100

§ i.e. The grower's mark.

It is clear that (*a*), the fruit does not ripen to any significant extent at 45° F. (corresponding to a cold spell in the market) ; (*b*), it ripens rapidly at 66° F. ; and (*c*), ethylene does not accelerate ripening at the higher temperature.

The fruit ripened in the boxes behaved similarly but, in 6 days at 66° F., two marks of the four examined had developed rotting of commercial significance (9 per cent. and 15 per cent. respectively). This may always be a risk and is probably due to the lack of uniformity of ripeness of the fruit on arrival. The initially ripe fruit would be expected to rot quickly at high temperature. Thus, boxes with 45 per cent., 57 per cent., 60 per cent. of ripe fruit developed 4 per cent., 9 per cent., and 15 per cent. of rotting in 6 days at 66° F.

The second series of experiments was done on end-of-season fruit, purchased in the market on 5th May. It was found :

(*a*) that ethylene did not accelerate the ripening of the fruit at 65° F. All the green tomatoes ripened in 19 days, and the turning fruits in 8 to 10 days. The initially ripe fruits rotted rapidly at 65° F.

(*b*) that the tomatoes did not ripen at 45° F. but that they ripened slowly at 50° F.

(*c*) that tomatoes held at 45° F. for more than ten days, subsequently failed to ripen satisfactorily at 65° F. but developed severe rotting. This is in accord with previous results on storage of tomatoes at low temperature.

The conclusions are that backward Canary Island tomatoes can be ripened at 65° F. but with risk of rotting of ripe fruits unless the packages are sorted. Ethylene is not beneficial. There is not much possibility of extending the season by cool storage, except for about two weeks, at 50° F.

DISCUSSION.

The data show that "Stonor's M.P." tomatoes, grown in a glasshouse, may be ripened in reasonably short time by storage in air at 65°-70° F. at high humidity, but that the time taken for outdoor grown tomatoes to ripen may be more than 3 weeks. Possibly this is not disadvantageous, since the logical way to make use of an artificial process is to use it on end-of-season fruit, to extend the season and to recover part of the unripe crop left on the vines. So long as the fruit will ripen on the vines, it will presumably reach optimum quality if it is not picked until it is ripe.

The results of the experiments where tomatoes were stored in air containing ethylene are puzzling and, in the light of present knowledge, it appears to be impossible to predict if an effect can be expected or not. The presentation of results under (*a*) (i), (ii) and (iii), lists some sixteen separate treatments of samples of tomatoes with ethylene. Five of these gave acceleration of ripening of all the fruit (including three experiments where the result was not clear cut), and two gave results similar to those obtained by Furlong, i.e. the ripening of only part of the sample was accelerated. There were nine treatments in which ripening proceeded at the same rate in the presence or absence of ethylene. These included all the later experiments, on fruit picked in October.

Thus the use of ethylene appears to be beneficial to less than half the samples treated and, if artificial ripening is used as suggested, at the end of season, it appears to confer no benefit.

Similar results were obtained with tomatoes from the Canary Islands, it being shown that the green and turning fruits can be ripened at 65° F., and that ethylene does not accelerate ripening.

The tomato thus appears to be a fruit which, like most varieties of plum imported from South Africa (but not the Kelsey variety), does not respond to ethylene.

SUMMARY.

1. English glasshouse and outdoor tomatoes may be ripened to good quality, from the green or turning stage, by holding at 65°-70° F. at high relative humidity.

2. The rate of ripening may sometimes be accelerated by the use of ethylene, but this gas usually produces very little or no effect.

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LABORATORY STUDIES ON THE TOXICITY OF HYDROCARBON OILS AND SIMILAR SUBSTANCES TO THE EGGS OF SOME COMMON ORCHARD PESTS

III. EXPERIMENTS ON THE EGGS OF THE GREEN APPLE APHIS (*Aphis pomi* Deg.)

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INTRODUCTION.

It is well known that, of the hydrocarbon oils commonly employed as constituents of winter ovicidal washes, those derived from the distillation of coal tar are toxic to eggs of aphides and psyllids, whereas petroleum distillates are effective against the eggs of capsids, lepidopterous pests, and the Fruit Tree Red Spider Mite, *Metatetranychus ulmi* (Koch). The literature on the relation between the toxicity of these oils and their physical and chemical properties was surveyed in detail by Martin (1935) who drew his data from laboratory and field experiments on a wide variety of insects and was led, so far as tar distillates are concerned, to the following conclusions :—

(*a*) High-boiling fractions are more toxic than those of low distillation range.

(*b*) Toxicity is associated with the proportion of the neutral oil soluble in dimethyl sulphate, a characteristic regarded as being a measure of the content of aromatic hydrocarbons in the oil.

(*c*) The source of the tar oil is relatively unimportant provided that the different oils have similar characteristics, especially distillation range and solubility in dimethyl sulphate.

(*d*) The phenolic components of the oil affect toxicity only in that they may act as diluents of the active constituents.

(*e*) The solids deposited from creosote and anthracene oils on cooling are relatively non-toxic.

(*f*) Insufficient evidence was available to assess the role of the tar bases.

Subsequently, Kearns, Martin and Wilkins (1937) examined the applicability of some of the above conclusions to results obtained with the eggs of *Aphis pomi* Deg. They confirmed the greater toxicity of high-boiling fractions and devoted considerable attention to the value of solubility in dimethyl sulphate as a criterion of toxicity, concluding :—

(*a*) That there is a " high degree of correlation . . . between the ovicidal properties of the washes and their content of neutral oils soluble in dimethyl sulphate."

(*b*) That " if high boiling tar oils exist which show ovicidal properties markedly inferior or superior to that represented by the dimethyl sulphate figure, it is probable that such oils would have been recognized in practical use. As no such cases have emerged it may be considered that the dimethyl sulphate test provides an adequate practical method of assessing the toxicity of high-boiling hydrocarbon oils to eggs of *A. pomi*."

(*c*) That the toxicity of the oils cannot be ascribed to any one of the series of pure chemical constituents of coal tar which they examined.

In arriving at conclusions (*a*) and (*b*) these authors had little evidence available for tar oils outside the range of high-temperature carbonization distillates normally used in tar oil winter washes. Moreover, their results with the hydrogenated tar oils tested were not in accord with these conclusions, for very high kills were obtained despite the relatively low content of neutral oil soluble in dimethyl sulphate. The issue was confused, however, by these oils having unusually high distillation ranges. Conclusion (*c*) has been confirmed for an extended series of pure compounds by Shaw and Steer (1939) and by later, unpublished work of Shaw, Steer, Tuft, and Davies at East Malling.

There is no evidence that the standardization of tar oil washes can, as yet, be based on anything but observed correlations between toxicity and the overall physical and chemical characteristics of the oils. The present paper reports the investigation of such possible correlations for a wider variety of hydrocarbon oil types than has hitherto been examined, using the eggs of *A. pomi* as test organism and bearing in mind the stress laid by previous workers on distillation range and solubility in dimethyl sulphate.

The characteristics determined were those in current use in oil specifications. Only when the work was well advanced was the application of such properties as specific refraction, specific dispersion, and " average molecular weight " developed to throw light on the molecular constitution of hydrocarbon oils. In the hands of American workers (Pearce, Chapman and Avens, 1942; Chapman, Pearce and Avens, 1942) these characteristics have since been used with great promise to evaluate petroleum oil types in relation to ovicidal action.

In the present investigation the following information was sought :—

(*a*) The precise location of maximum toxicity in the distillation range.

(*b*) The relative toxicity of different oil types of broadly similar physical properties.

(*c*) The value of dimethyl sulphate solubility as a criterion of toxicity.

(*d*) The role of tar phenols and tar bases in determining toxicity.

(*e*) The possibility of joint action in mixtures of different oil types.

(*f*) The relation of susceptibility to the physiological state of the developing egg as determined by varying the date of application of the ovicide.

EXPERIMENTAL.

Materials and methods.

Chemical. The following oils were used in the tests described in this paper, the abbreviations employed later in the text being indicated in parentheses: Horizontal Retort High-Temperature Tar Distillate (H.T.), Vertical Retort High-Temperature Tar Distillate (V.T.), Vertical Retort Low-Temperature Tar Distillate (L.T.), Hydrogenated Low-Temperature Tar (Hydr. L.T.), Hydrogenated Coal (Hydr. Coal), Creosote Oil (Creos.), Spindle Oil (Spin.), and an Edeleanu extract (Edel.). General descriptions of these oils have been given by Shaw and Steer (1947a) in Part I of this series where also the techniques used for fractionation, determination of characteristics, and emulsification of the oils have been described. As in Part II (Shaw and Steer, 1947b), the percentage composition of the oils and all concentrations in the tables of results are expressed by volume unless stated otherwise.

Biological. The methods used in obtaining supplies of *A. pomi* eggs and in conducting the tests have been described by Steer (1938). As the susceptibility of the eggs varies somewhat from year to year and also depends on the date on which the test is conducted, the different tests in a comparative experiment were always carried out at the same time. Comparisons between tests made on different occasions may result in misleading inferences and have been avoided throughout this paper except for the semi-quantitative demonstration of very large differences. In all tables of results a code number is quoted for each test, indicating the season of testing ; thus, 13/38 denotes test 13 of the winter 1938-39. The appropriate control series can, therefore, be identified readily in the tables.

Statistical analysis of data. The mean percentage hatch and its standard error have, in general, been calculated from the five replicates of each treatment. Whenever practicable the experiments were so planned as to permit interpretations in terms of the dosage-mortality regression equation and statistics derived from it, such as median lethal dose and relative potency. Computational techniques follow those given by Finney (1947) and are improvements on the methods due to Bliss (1935a, 1935b). In a few cases it was possible to conduct more restricted experiments only and the methods of statistical analysis used in these instances are described at the appropriate point in the text.

PRELIMINARY SURVEY.

In order to survey the biological performance of all the oils on which detailed investigations were projected, a preliminary examination was conducted during 1936 and 1937. In this, no attempt was made to locate that part of the distillation range showing maximum toxicity, the wide fraction boiling above about 250° C. or the whole oil as received being tested. The biological results obtained, together with the relevant physical data, are presented in Table I, from which it is immediately apparent that the various oil types differ considerably in their ovicidal power. The first conclusion which emerges from the results is that the highly paraffinic spindle oil is of very low toxicity and the Hydr. L.T., consisting mainly of naphthenic and mixed paraffinic-naphthenic types, is only a little more toxic. On the other hand, the predominantly aromatic oils—H.T., V.T., L.T., and Hydr. Coal—are markedly toxic, though V.T. and, more particularly, L.T. are less so than H.T. and Hydr. Coal. So far as the neutral oils are concerned, the order of toxicity for the three tar distillates is H.T. > V.T. > L.T. and a similar relationship holds for the corresponding whole oils examined in test 17/37. Disagreement between tests 6/36 and 17/37, however, renders some of the conclusions of doubtful validity. There is no very obvious evidence of toxicity being correlated with any single characteristic of the oils, though there is some support for the view that distillation range, taken in conjunction with solubility in dimethyl sulphate, provides a guide to toxicity. Thus, in the three neutral oils of test 6/36 the most toxic (H.T.) is completely soluble and has a large proportion of high-boiling constituents while the difference between V.T. and L.T., which respond similarly to the dimethyl sulphate test, is explicable by the higher distillation range of the former. Again, although 20 per cent. of the Hydr. Coal

TABLE I.

Toxicity of wide fractions of oils.

Test. No.	Date of treatment.	Oil.	Conc. %	Biological data.			Characteristics of oils.				Distillation range in ° C.			
				Mean % hatch.	S.E.	Total No. of eggs.	Neutral oil, %	Sp. gr.	U.S.R. %	Insol. in Me_2SO_4, %	10%	50%	80%	>300°C.
6/36	17.2.37	H.T. neutral > 250° C.	2·0	2·2	0·18	1,731	97·7	1·084	0	0	242	306	354	53%
			1·0	7·0	1·22	1,817								
			0·5	41·1	1·85	806								
6/36	17.2.37	V.T. neutral > 250° C.	2·0	19·3	4·47	1,310	98·4	1·012	20	33	272	306	342	56%
			1·0	32·0	0·59	2,039								
			0·5	63·3	5·27	1,266								
6/36	17.2.37	L.T. neutral > 250° C.	2·0	43·1	3·22	2,842	98·4	0·994	18	34	245	291	332	44%
			1·0	50·2	0·83	1,487								
			0·5	67·9	4·73	1,403								
6/36	17.2.37	Hydr. L.T. whole > 250° C.	2·0	49·4	0·73	1,190	99·2	0·979	18	66	264	313	356	58%
			1·0	83·0	2·78	1,666								
6/36	17.2.37	H.T. whole > 250° C.	1·0	56·3	1·05	1,202	84·5	1·034	4	0	224	273	330	38%
6/36	17.2.37	V.T. whole > 250° C.	1·0	17·6	1·03	2,091	88·0	1·010	17	26	270	306	344	55%
6/36	17.2.37	L.T. whole > 250° C.	1·0	39·5	1·60	2,134	72·1	0·986	14	30	234	286	327	43%
8/36	23.2.37	Spin.	3·6	64·6	1·94	1,536	100·0	0·901	72	96	347	368	389	100%
8/36	23.2.37	Hydr. Coal whole ..	2·0	0·0	—	1,336	96·1	1·082	—	20	—	—	—	98%
			1·0	1·7	0·10	1,944								
17/37	3.3.38	Edel.	7·7	44·1	7·62	2,383	97·2	0·997	16	22	321	357	390	94%
8/36	23.2.37	Creos.	2·0	23·2	1·33	1,354	81·3	1·023	0	8	221	262	354	30%
			1·0	38·8	1·26	790								
17/37	3.3.38	H.T. whole > 250° C.	1·0	27·7	5·35	2,515	84·6	1·034	4	0	224	273	330	38%
17/37	3.3.38	V.T. whole > 250° C.	1·0	52·9	10·45	2,348	88·0	1·010	17	26	270	306	344	55%
17/37	3.3.38	L.T. whole > 250° C.	1·0	76·9	9·62	1,381	72·1	0·986	14	30	234	286	327	43%
17/37	3.3.38	Hydr. L.T. whole > 250° C.	1·0	68·8	10·67	2,305	99·2	0·979	18	66	264	313	356	58%
6/36	17.2.37	Control	—	83·7	3·94	1,565								
8/36	23.2.37	Control	—	94·2	0·50	1,646								
8/36	23.2.37	Soap control	1·0	88·4	3·88	1,443								
17/37	3.3.38	Control	—	93·5	2·57	2,170								

TABLE II.

Characteristics of neutral oil fractions.

Distillation range in °C.	Type.	Viscosity in secs. Redwood I at 70° F.	Insoluble in Me$_2$SO$_4$, %
100-150	V.T.	—	—
150-200	H.T.	—	0
	V.T.	—	32
	Creos.	—	22
200-225	H.T.	—	0
	V.T.	—	30
	Creos.	—	24
225-250	H.T.	—	0
	V.T.	—	28
	Creos.	—	26
250-275	H.T.	—	0
	V.T.	—	22
	L.T.	38	28
	Hydr. L.T.	35	68
	Creos.	—	24
275-300	H.T.	—	0
	V.T.	—	26
	L.T.	45	30
	Hydr. L.T.	41	70
	Creos.	—	30
300-325	H.T.	—	0
	V.T.	—	30
	L.T.	63	30
	Hydr. L.T.	55	68
	Creos.	—	32
	Hydr. Coal	74	30
325-350	H.T.	—	0
	V.T.	—	26
	L.T.	130	30
	Hydr. L.T.	97	66
	Creos.	—	34
	Hydr. Coal	151	26
350-375	H.T.	—	0
	V.T.	—	24
	L.T.	525	26
	Hydr. L.T.	262	70
	Creos.	—	32
	Hydr. Coal	422	20
375-400	H.T.	—	0
	V.T.	—	—
	L.T.	3,692	22
	Hydr. L.T.	—	54
	Creos.	—	—
	Hydr. Coal	2,190	28
400-415	L.T.	—	—
400-425	Hydr. Coal	13,500	—
> 425	Hydr. Coal	—	—
300-350	Edel.	121	72
350-400	Edel.	981	80
350-400	Spin.	185	94

TABLE III.

Toxicity of narrow fractions of neutral oils.

Test No.	Date of treatment.	Distillation range in ° C.	Type.	Concentration of oil.								
				2·0%			1·0%			0·5%		
				Mean % hatch.	S.E.	Total No. of eggs.	Mean % hatch.	S.E.	Total No. of eggs.	Mean % hatch.	S.E.	Total No. of eggs.
13/37	23.2.38	100-150	V.T.	81·3	3·17	2,167	—	—	—	—	—	—
13/37	23.2.38	150-200	V.T.	86·6	1·16	2,129	—	—	—	—	—	—
7/37	21.2.38		H.T.	46·9	1·43	4,423						
10/37	22.2.38		Creos.	79·2	6·54	1,940						
7/37	21.2.38	200-225	H.T.	60·0	4·04	2,171	67·2	6·57	1,695	—	—	—
13/37	23.2.38		V.T.	86·3	1·74	1,645	—	—	—			
10/37	22.2.38		Creos.	83·3	1·65	1,737	—	—	—			
7/37	21.2.38	225-250	H.T.	50·7	1·33	2,616	65·4	2·82	2,513	—	—	—
13/37	23.2.38		V.T.	58·7	1·51	2,497	—	—	—			
10/37	22.2.38		Creos.	88·5	2·53	1,584	94·4	—	415*			
7/37	21.2.38	250-275	H.T.	16·6	1·97	3,653	36·1	2·05	2,548	—	—	—
13/37	23.2.38		V.T.	63·4	3·17	1,855	76·9	4·81	1,523			
12/37	23.2.38		L.T.	62·0	5·01	2,277	—	—	—			
10/37	22.2.38		Hydr. L.T.	53·8	2·28	1,826	—	—	—			
10/37	22.2.38		Creos.	87·3	1·15	2,196	76·0	—	538*			
7/37	21.2.38	275-300	H.T.	9·0	0·68	2,304	38·5	4.86	2,433	—	—	—
13/37	23.2.38		V.T.	34·4	5·20	1,426	73·8	2·20	1,590			
12/37	23.2.38		L.T.	61·6	1·46	2,510	—	—	—			
10/37	22.2.38		Hydr. L.T.	46·5	2·16	2,890	—	—	—			
11/37	22.2.38		Creos.	77·8	3·01	2,215	82·3	1·15	896**			
7/37	21.2.38	300-325	H.T.	1·9	0·52	2,861	5·4	1·08	2,710	42·0	2·09	3,500
14/37	25.2.38		V.T.	47·8	1·55	1,685	33·4	0·87	1,987	58·1	1·84	1,628
12/37	23.2.38		L.T.	43·5	2·28	2,370	64·6	1·34	2,901	—	—	—
10/37	22.2.38		Hydr. L.T.	49·4	3·23	1,980	—	—	—	—	—	—
11/37	22.2.38		Creos.	38·4	13·50	1,771	67·3	3·88	2,856	92·7	1·48	1,807
14/37	25.2.38		Hydr. Coal	3·5	2·55	3,134	26·9	2·59	2,067	59·7	4.69	1,891
7/37	21.2.38	325-350	H.T.	0·02	0·02	2,764	3·7	1·28	1,880	28·1	4·96	2,976
14/37	25.2.38		V.T.	0·2	0·20	2,303	4·8	1·22	2,828	41·3	1·98	1,694
12/37	23.2.38		L.T.	39·4	2·39	1,854	69·3	2·82	2,191	—	—	—
10/37	22.2.38		Hydr. L.T.	9·3	2·04	2,664	—	—	—	—	—	—
11/37	22.2.38		Creos.	24·2	1·53	1,827	41·8	4·46	2,103	72·9	2·84	2,098
15/37	28.2.38		Hydr. Coal	0·3	0·36	2,779	17·8	0·47	1,782	30·7	0·81	1,823
8/37	21.2.38	350-375	H.T.	0·0	—	2,427	0·9	1·40	2,444	9·8	7·52	2,546
14/37	25.2.38		V.T.	0·0	—	1,795	1·6	0·46	2,195	28·8	1·10	2,270
12/37	23.2.38		L.T.	4·9	1·25	2,406	58·3	2·54	2,530	85·1	5·37	2,072
10/37	22.2.38		Hydr. L.T.	2·4	2·36	1,983	—	—	—	—	—	—
11/37	22.2.38		Creos.	22·3	7·42	2,528	63·5	6·56	2,729	82·9	1·59	2,403
15/37	28.2.38		Hydr. Coal	0·0	—	2,733	9·0	4·19	1,641	81·4	3·79	1,576
8/37	21.2.38	375-400	H.T.	0·0	—	2,766	0·2	0·07	2,262	16·0	3·72	2,075
14/37	25.2.38		V.T.	0·7	0·31	2,138	3·4	0·63	2,991	22·3	4·40	3,134
12/37	23.2.38		L.T.	34·1	2·28	2,855	68·8	2·19	1,926	80·8	1·51	2,259
10/37	22.2.38		Hydr. L.T.	1·6	0·32	2,244	—	—	—	—	—	—
11/37	22.2.38		Creos.	49·1	2·02	2,091	62·8	3·40	1,976	81·2	2·74	1,743
15/37	28.2.38		Hydr. Coal	4·1	0·47	2,265	8·4	2·48	2,373	82·2	2·52	1,867
12/37	23.2.38	400-415	L.T.	40·7	1·66	2,577	74·1	1·28	1,562	96·4	1·00	2,073
15/37	28.2.38	400-425	Hydr. Coal	0·7	0·15	3,004	6·2	1·49	2,421	11·1	2·00	3,564
15/37	28.2.38	> 425	Hydr. Coal	12·1	0·74	2,439	25·5	4·86	2,233	72·1	2·43	1,651

* 1 replicate.
** 2 replicates.

Controls : 7/37 96·4 ± 0·93 (3,392).
8/37 96·4 ± 0·93 (3,392).
10-12/37 95·1 ± 1·22 (1,345).
14/37 92·1 ± 1·77 (1,271).
15/37 94·3 ± 1·49 (3,311).

is insoluble in dimethyl sulphate, it seems that its very high distillation range compensates for that fact and gives it excellent ovicidal properties while the poor performance of Spin., which also has a high distillation range, is associated with its almost complete insolubility in dimethyl sulphate. The extent to which these two characteristics of the oils account for their toxicity is examined in greater detail in later sections of this paper.

DISTILLATION RANGE, OIL TYPE AND TOXICITY.

Tests of narrow fractions. A series of narrow fractions taken over most of the distillation range of the neutral oils of seven oil types was next examined with the object of locating the fractions of greatest ovicidal activity. The available characteristics of these fractions are given in Table II, while the biological results—obtained mainly in tests using oil concentrations of 2·0, 1·0 and 0·5 per cent.—are shown in Tables III and IV.

TABLE IV.

Toxicity of petroleum oil fractions.

Test No. 9/37. Date of treatment 21.2.38.

Distillation range in °C.	Type.	Concentration of oil.								
		3·0%			2·0%			1·0%		
		Mean % hatch.	S.E.	Total No. of eggs.	Mean % hatch.	S.E.	Total No. of eggs.	Mean % hatch.	S.E.	Total No. of eggs.
300-350	Edel.	20·1	4·90	1,688	65·0	2·43	3,878	65·7	3·51	4,072
350-400	Edel.	57·7	3·52	5,916	70·4	8·83	4,721	—	—	—
350-400	Spin.	81·1	1·99	4,893	68·0	1·04	5,033	—	—	—

Control: 85·7 ± 3·21 (5,557).

The data embodied in the above tables have been summarized in Table V in which the various oil fractions have been arranged in three groups in order of toxicity. Group I includes those fractions permitting up to about 10 per cent. hatch at 2·0 per cent. concentration; Group II consists of those giving 10-60 per cent. hatch while Group III comprises those allowing over 60 per cent. hatch. This table shows convincingly the poor performance of the petroleum oils (Spin. and Edel.), L.T., and Creos. on the one hand and the high toxicity of H.T. and Hydr. Coal on the other, with the V.T. and Hydr. L. T. displaying intermediate values. It also brings out the failure of distillation range alone to account for the differences in toxicity between different oil types. Thus the H.T. fractions between 275° C. and 400° C. are all in Group I, whereas similar Creos. fractions are found in Groups II and III; again, the 350-375° C. fractions of H.T., Hydr. Coal, V.T., Hydr. L.T. and L.T. are all in Group I while the corresponding Spin. fractions are in Group III.

Graphs relating percentage hatch to distillation range are shown in Figs. 1 and 2 and make clear the increase in toxicity with increasing distillation range up to 350-375° C., after which there is in some cases (e.g. L.T., Creos. and Hydr. Coal at 2·0 per cent. and L.T. at 1·0 per cent.) a decline in toxicity. Certainly it is true that for every oil type graphed, maximum toxicity is associated with the fractions distilling above 325° C., but it is equally clear that for any given distillation range there are wide differences in the toxicity of the different oil types. On the other hand, these differences are not closely correlated with solubility in dimethyl sulphate, as can readily be seen from those data of Tables II and III which have been selected and rearranged in Table VI. In each group of 25° C. fractions from 275° C. to 400° C. the order of toxicity differs from the order of solubility in dimethyl sulphate, the only exception being the H.T. oil which consistently heads each list.

TABLE V.

Miscellaneous oil fractions arranged in order of toxicity.

Group.	Oil.	Fraction.
I.	H.T.	All fractions from 275°C. to 400°C.
	Hydr. Coal	,, ,, ,, 300°C. to 425°C.
	V.T.	,, ,, ,, 325°C. to 400°C.
	Hydr. L.T.	,, ,, ,, 325°C. to 400°C.
	L.T.	350°C. to 375°C.
II.	H.T.	150-200°C. ; 250-275°C.
	V.T.	All fractions from 275°C. to 325°C.
	Hydr. L.T.	,, ,, ,, 275°C. to 325°C.
	L.T.	300-350°C. ; 375-400°C.
	Creos.	All fractions from 300°C. to 400° C.
	Hydr. Coal	> 425°C.
III.	V.T.	All fractions from 100°C. to 275°C.
	H.T.	200°C. to 250°C.
	Hydr. L.T.	All fractions below 275°C.
	L.T.	,, ,, ,, 300°C.
	Creos.	,, ,, ,, 300°C.
	Edel.	All fractions.
	Spin.	,, ,,

TABLE VI.

Toxicity and Me_2SO_4-solubility of 2 per cent. concentrations of neutral oils.

Oil.	Distillation range.							
	300-325°C.		325-350°C.		350-375°C.		375-400°C.	
	Mean % hatch.	Insol. in Me_2SO_4 %	Mean % hatch.	Insol. in Me_2SO_4 %	Mean % hatch.	Insol. in Me_2SO_4 %	Mean % hatch.	Insol. in Me_2SO_4 %
H.T.	1·9	0	0·02	0	0·0	0	0·0	0
V.T.	47·8	30	0·2	30	0·0	26	0·7	—
L.T.	43·5	30	39·4	26	4·9	24	34·1	22
Hydr. L.T. ..	49·4	68	9·3	66	2·4	70	1·6	54
Creos.	38·4	32	24·2	34	22·3	32	49·1	—
Hydr. Coal ..	3·5	30	0·3	26	0·0	20	4·1	28

TABLE VII.

Characteristics of oil fractions distilling 320° C. to 380° C.

Oil.	Sp. gr.	Viscosity in secs. Redwood I at 70° F.	Insoluble in Me_2SO_4 %	U.S.R. %
H.T. neutral ..	1.109	128	0	0
L.T. neutral ..	1.026	168	28	15
Spin.	0.894	112	92	62

TABLE VIII.

Toxicity of five neutral oils of common distillation range 320° C. to 380° C.
Date of treatment :. 19.1.43.

Test No.	Oil.	Conc. %	Mean % hatch.	S.E.	Total No. of eggs.	Kill, % (Control = 0·0).
8/42	H.T.	2·0	0·0	—	2,880	100·0
		1·0	4·7	2·8	2,337	92·6
		0·5	34·9	9·9	3,540	44·8
		0·25	57·6	4·6	2,788	9·9
		0·125	60·0	2·3	2,507	5·1
8/42	V.T.	4·0	0·0	—	2,940	100·0
		2·0	2·8	1·6	3,404	95·6
		1·0	31·2	4·1	2,816	50·6
		0·5	52·7	6·2	3,795	16·6
		0·25	61·8	7·9	3,511	2·2
8/42	L.T.	8·0	0·3	0·3	1,738	99·5
		4·0	2·7	1·5	2,921	95·7
		2·0	33·5	5·4	2,739	47·0
		1·0	49·8	3·5	3,334	21·2
		0·5	65·0	7·8	3,288	0·0
9/42	Hydr. L.T.	8·0	0·0	—	2,747	100·0
		4·0	10·2	4·2	4,863	83·9
		2·0	38·7	8·7	2,777	38·8
		1·0	48·7	2·6	3,122	22·9
		0·5	58·8	4·3	3,699	7·0
9/42	Spin.	8·0	37·8	4·4	3,344	40·2
		4·0	50·5	5·5	3,258	20·1
		2·0	60·0	4·4	2,768	5·1
		1·0	59·4	2·3	2,447	6·0
		0·5	62·6	3·0	2,655	1·0
8-9/42	Control	—	58·3	5·1	3,414	—
8-9/42	Soap control	—	63·2	2·2	2,633	—

Tests of wider fractions. The data in Tables III and IV are hardly suitable for a precise quantitative estimate of the extent to which similar fractions of the various oil types differ in toxicity, since too small a range of concentrations is available in many cases. Accordingly, a detailed comparison of five important oil types—H.T., V.T., L.T., Hydr. L.T. and Spin.—was made, using 60° C. fractions of the neutral oils in the region of maximum toxicity, i.e. 320° C. to 380° C. The available characteristics are given in Table VII and the results of the biological test in Table VIII.

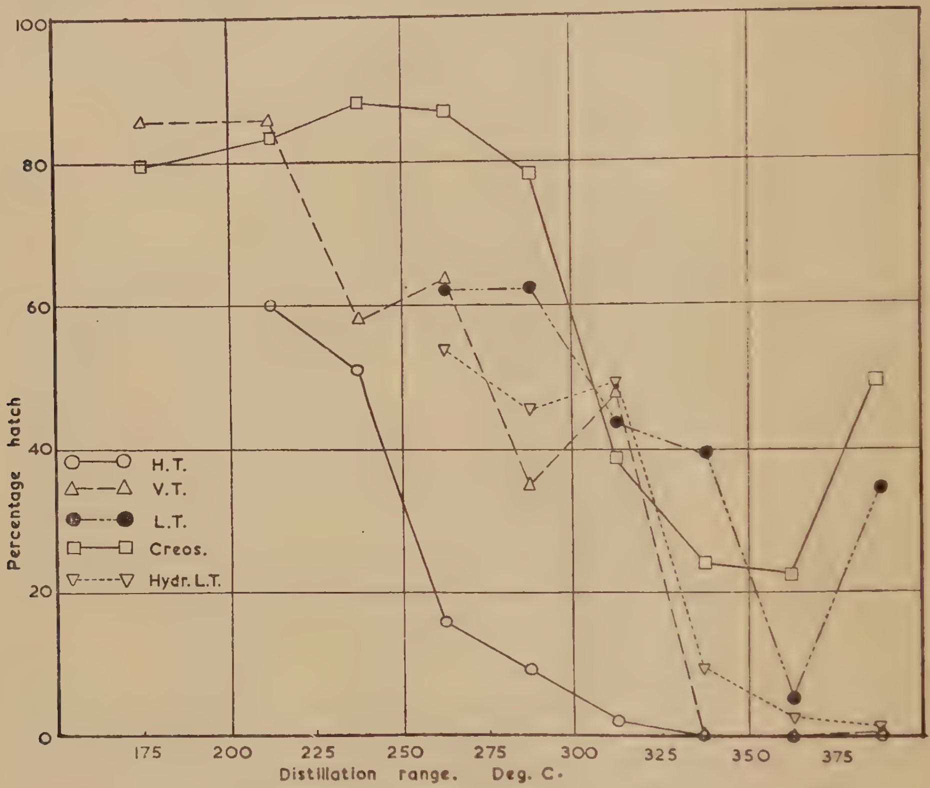

FIG. 1.

Mean percentage hatch plotted against distillation range. All oils tested at 2·0 per cent. concentration. The abscissa of each point is the mid-point of the distillation range.

From the latter the log-probit regression equations were calculated and, taking the H.T. regression line as a standard, the lines for the remaining oils were compared with it by the t-tests of Bliss (1935b). In no case did the lines differ significantly ($P = 0\cdot05$) in slope but each line differed significantly from the standard in position. It is, therefore, valid to compare the data in terms of the relative potencies of the oils concerned and these have been calculated by the methods of Cochran (1938) to give results shown in Table IX. As data on the dimethyl sulphate solubilities of these particular samples are incomplete, they cannot be used to decide the extent to which the differences are explicable in terms of aromatic content, but it is interesting to note that the dimethyl sulphate solubility figure for L.T. would suggest it to be about 0·8 times as toxic as H.T. on the assumption that non-aromatic constituents are inactive, whereas, in fact, it is only about 0·3 times as toxic. Against this, however, is the fairly good agreement between the dimethyl sulphate solubility figure for Spin. and its actual relative potency.

The results of an earlier experiment (part of Test 6/36 of Table I) comparing H.T., V.T., and L.T. by means of their neutral oils distilling above 250° C. were now analysed in the same way. This experiment was not entirely satisfactory as the variability of the biological material prevented the demonstration of a significant difference in the position of the probit-log. dosage regression lines for H.T. (standard) and V.T. Nevertheless, the large and significant difference in the relative potency of L.T. compared with H.T. is evident from the summarized analyses of Table X, while a consideration of the ratio of median lethal doses of H.T. and V.T. leads to a conclusion similar to that derived from the results with the narrower fractions of the previous experiment. Summarizing, one may say that for fractions of similar distillation range (320° C. to 380° C.) H.T. neutral oil is about twice as toxic as V.T. neutral,

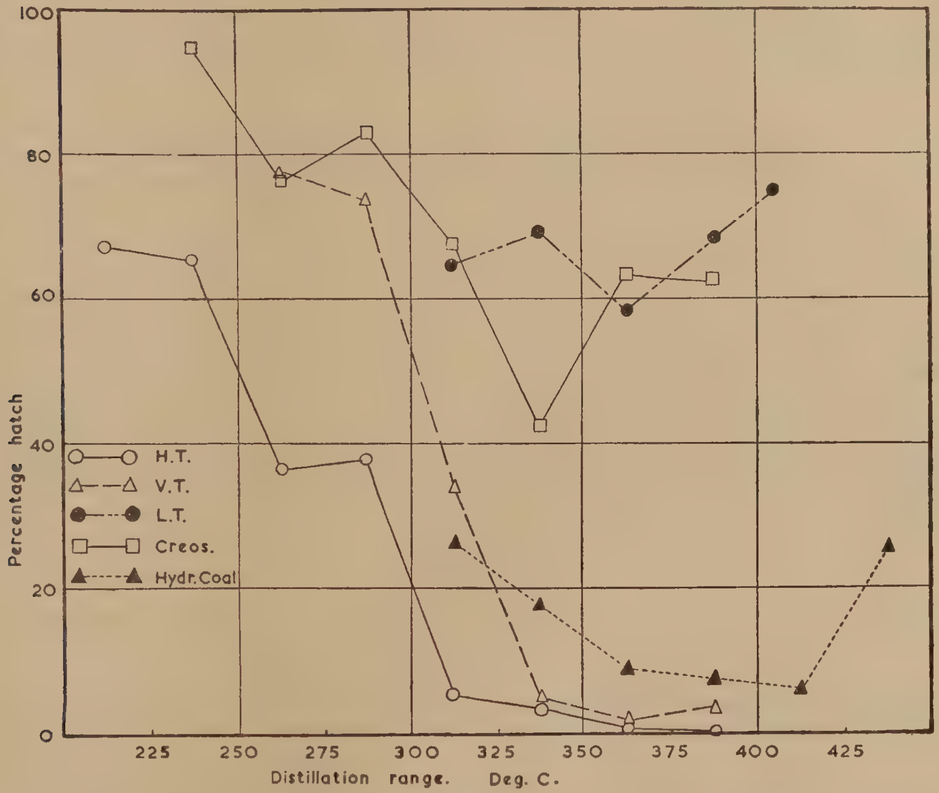

FIG. 2.

Mean percentage hatch plotted against distillation range. All oils tested at 1·0 per cent. concentration. The abscissa of each point is the mid-point of the distillation range.

Table IX.

Relative toxicity of oil fractions, 320-380° C.

Oil.	Regression equation.	LD50.	Relative Potency.
H.T.	$Y = 3\cdot93x + 2\cdot27$	0·49%	1·0
V.T.	$Y = 4\cdot46x + 0\cdot74$	0·90%	0·55 ± 0·11
L.T.	$Y = 4\cdot30x - 0\cdot44$	1·84%	0·33 ± 0·08
Hydr. L.T.	$Y = 3\cdot05x + 1\cdot04$	1·99%	0·25 ± 0·06
Spin.	$Y = 2\cdot08x + 0\cdot79$	10·45%	0·06 ± 0·01

Note.—In these and subsequent regression equations, Y = mortality expressed in probits and x = log. (dose x 10).

Table X.

Relative potencies of neutral oils distilling above 250° C.

Oil.	Regression Equation.	LD50.	Relative Potency.
H.T.	$Y = 3\cdot40x + 2\cdot78$	0·45%	1·0
V.T.	$Y = 2\cdot28x + 2\cdot88$	0·84%	—
L.T.	$Y = 1\cdot26x + 3\cdot35$	2·04%	0·17 ± 0·06

about four times as toxic as L.T. neutral and Hydr. L.T. neutral, and about fifteen times as toxic as the spindle oil.

Broad cuts of H.T. Neutral. Fractions of the type considered in detail in earlier sections are too narrow to be considered from the commercial aspect, but in view of the toxicity maximum in the range 325° C. to 375° C. it was considered worth while to compare quantitatively three broad cuts (> 200° C., > 300° C. and > 350° C.) of the most toxic oil (H.T. neutral). The biological data are given in Table XI.

Table XI.

Toxicity of wide cuts of a horizontal-retort neutral tar oil.
Test No. 7/38. Date of treatment: 3.2.39.

Conc. %	Distilling > 200° C.			Distilling > 300° C.			Distilling > 350° C.		
	Mean % hatch.	S.E.	Total No. of eggs.	Mean % hatch.	S.E.	Total No. of eggs.	Mean % hatch.	S.E.	Total No. of eggs.
1·5	17·2	3·84	1,958	0·0	—	1,781	1·0	0·36	1,934
1·0	32·4	3·50	2,190	4·4	2·03	1,695	2·1	0·91	1,992
0·75	49·9	0·90	2,568	16·4	2·48	1,746	3·7	0·50	1,945
0·5	55·5	2·33	2,165	38·3	3·09	1,365	22·6	3·30	2,039
0·25	56·0	4·57	2,991	54·4	7·87	1,665	47·6	1·73	2,255

Controls: 92·1 ± 2·72 (1,879).

Statistical analysis yields three regression lines:

(i) > 350° C. $Y = 3\cdot28x + 3\cdot60$ LD50 = 0·27 per cent.
(ii) > 300° C. $Y = 3\cdot14x + 3\cdot32$ LD50 = 0·34 per cent.
(iii) > 200° C. $Y = 1\cdot33x + 4\cdot00$ LD50 = 0·56 per cent.

Lines (i) and (ii) are similar in slope and position. Lines (i) and (iii) differ significantly in both slope and position so that no comparison in terms of relative potency is possible. Lines (ii) and (iii) are similar in slope but differ in position and the relative potency of the > 200° C. cut compared with the > 300° C. one is 0·43 ± 0·14. It seems, therefore, that the effect of retaining the lower-boiling fractions of H.T. neutral is to reduce the toxicity appreciably, but that the fraction distilling above 300° C. is virtually as toxic as that distilling above 350° C.

TOXICITY AND SOLUBILITY IN DIMETHYL SULPHATE.

Kearns, Martin and Wilkins (1937) found " no evidence of large differences in the ovicidal properties of high-boiling neutral oils soluble in dimethyl sulphate derived from various sources ". The range of oil types examined was comparatively restricted but even so these authors found and commented on certain divergences from this generalization. They considered these unimportant, however, and suggested that major divergences were not likely to be encountered among high-boiling tar oils. Hence the dimethyl sulphate test was considered to give an adequate assessment of the toxicity of any high-boiling hydrocarbon oil to *A. pomi* eggs. Figures quoted in earlier sections of the present paper cast further doubt on the validity of the generalization and the question was therefore examined in greater detail in three groups of experiments.

(i) A series of neutral oils distilling above 250° C. was tested at concentrations equivalent in each case to 2·0 per cent. and 1·5 per cent. of oil soluble in dimethyl sulphate. If dimethyl sulphate solubility were a sufficient criterion of toxicity irrespective of the source of the oil and if the insoluble fractions were virtually inert, then the percentage hatches at corresponding concentrations of the different oils might be expected to vary only within the limits of sampling error. The results obtained are shown in Table XII.

TABLE XII.

Toxicity of Neutral Oils of Equivalent Me_2SO_4-solubility.
Test No. 17/37. Date of treatment, 3.3.38.

Oil.	Conc. %	Equivalent conc. soluble in Me_2SO_4 %	Mean % hatch.	S.E.	Total number of eggs.
H.T. > 250° C. ..	2·0	2·0	1·1	0·59	3,248
	1·5	1·5	6·8	1·92	2,116
V.T. > 250° C. ..	2·7	2·0	10·2	2·49	2,170
	2·025	1·5	22·6	5·50	2,982
L.T. > 250° C.	2·87	2·0	56·5	2·47	3,041
	2·15	1·5	51·6	1·82	1,407
Hydr. L.T. > 250° C.	6·67	2·0	8·5	3·58	2,020
Hydr. Coal ..	3·23	2·0	0·0	—	2,074
	2·42	1·5	0·3	0·21	1,691
Edel.	7·7	2·0	44·1	7·62	2,383
Controls	—	—	82·5	6·43	1,304

These data were examined statistically by subjecting the untransformed data to a series of analyses of variance, incorporating full replication in the analysis. It may be pointed out that the inverse sine transformation of Bliss (1937) is unsuitable because the number of eggs used varies fairly widely from test to test so that variances cannot be equalized and also because information on the interaction of oil type with concentration was sought. The Hydr. Coal data were omitted from the analyses because their variation cannot be assumed to be normal and there is, therefore, no reliable test which can be used to compare them with the remaining data although this oil is apparently the most toxic. The analyses showed that the oil types interact significantly with concentration but that, of those tested at two concentrations, only the V.T. neutral gave a significantly higher hatch at 1·5 per cent. than at 2·0 per cent. Comparisons of the different oil types at 2·0 per cent. concentration showed that the hatches for H.T., Hydr. L.T. and V.T. did not differ significantly, but all were significantly more toxic than Edel. and L.T., which were again equal within the limits of sampling error. At 1·5 per cent. concentration each pair of tar oils differed significantly in toxicity, the order being H.T. > V.T. > L.T. It is evident, therefore, that the constituents soluble in dimethyl sulphate differ in their toxicity according to the source of the oil.

(ii) It might be argued that the above results were, in some cases, due to the dilution of the constant concentration of aromatic oil by varying concentrations of paraffinic, naphthenic and unsaturated hydrocarbons. The dimethyl sulphate-soluble fractions of a series of oils were, therefore, separated by alkaline hydrolysis

Table XIII.

Toxicity of neutral oil fractions soluble in Me_2SO_4.

Test No.	Date of treatment.	Parent oil.	Conc. %	Mean % hatch.	S.E.	Total number of eggs.
15/38	22.2.39	H.T. > 250° C.	1·5	0·1	0·12	1,964
			1·0	3·6	1·53	1,762
			0·75	13·1	2·74	1,599
			0·5	18·3	8·46	1,179
			0·25	49·9	3·03	1,621
15/38	22.2.39	V.T. > 250° C.	2·0	6·9	2·76	1,621
			1·5	17·9	2·70	1,822
			1·0	35·7	7·86	1,242
			0·75	43·7	4·11	1,238
			0·5	64·2	2·27	1,596
15/38	22.2.39	L.T. > 250° C.	3·0	35·5	3·38	1,567
			2·0	42·7	3·97	1,474
			1·5	61·6	3·84	1,422
			1·0	60·7	3·75	1,131
			0·75	79·3	2·66	1,540
16/38	22.2.39	Hydr. L.T.>250° C.	2·0	29·5	2·32	1,722
			1·5	53·9	7·01	2,163
			1·0	55·2	1·97	2,853
			0·75	72·8	8·00	1,769
			0·5	77·9	3·39	2,007
15-16/38	22.2.39	Controls	—	83·2	5·42	4,389

of the dimethyl sulphate extract and isolation of the liberated oil and were tested alone. The parent oils used and the results of the biological tests are given in Table XIII, while the statistical treatment of the results (in which the H.T. fraction was taken as the standard) is summarized in Table XIV. These tables demonstrate the fact that the aromatic constituents of H.T. are about three times as toxic as those of V.T., which, in turn, are about twice as toxic as those of L.T. and Hydr. L.T.

TABLE XIV.

Relative toxicity of neutral oil fractions soluble in Me_2SO_4.

Parent oil.	Regression equation.	LD50.	Relative potency.
H.T.	$Y = 3{\cdot}18x + 3{\cdot}46$	0·31%	1·0
V.T.	$Y = 3{\cdot}41x + 1{\cdot}87$	0·83%	0·38 ± 0·06
L.T.	$Y = 2{\cdot}01x + 2{\cdot}19$	2·50%	0·13 ± 0·04
Hydr. L.T.	$Y = 2{\cdot}83x + 1{\cdot}55$	1·66%	0·18 ± 0·04

(iii) Finally, the fractions insoluble in dimethyl sulphate were tested to decide whether the aliphatic components of the neutral oils contribute towards their toxicity. The biological results are given in Table XV, where it should be noted that the figures for V.T., L.T. and Hydr. L.T. are based on four replicates per concentration instead of the five usually employed.

TABLE XV.

Toxicity of neutral oil fractions insoluble in Me_2SO_4.
Test No. 16/38. Date of treatment, 22.2.39.

Parent oil.	4·0%			2·0%			0·6%		
	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.
V.T. > 250° C.	43·6	4·84	1,535	74·8	3·64	2,132	—	—	—
L.T. > 250° C.	44·6	4·87	1,947	78·5	9·63	1,599	—	—	—
Hydr. L.T. >250° C. ..	59·2	5·99	1,017	75·0	7·62	1,323	—	—	—
Spin.	53·6	2·48	1,786	83·4	5·08	1,795	77·2	5·12	1,460
Paraffin 200-250° C.	—	—	—	—	—	—	86·0	4·36	1,021
Paraffin ..	—	—	—	—	—	—	84·6	2·71	1,379

Controls: 79·8 ± 7·30 (3,399).

Statistical examination of these data by analysis of variance revealed that the oils tested by 4·0 per cent. and 2·0 per cent. showed the sole significant effect of concentration—the oil types did not differ significantly nor was there a significant interaction of oil type and concentration. It may be concluded, therefore, that while the aliphatic components of V.T., L.T., Hydr. L.T. and Spin. are slightly toxic, by far the greater part of the toxicity of the neutral oils is associated with the dimethyl sulphate-soluble fraction and the differences in the toxicity of the four neutral oils from different sources (H.T., V.T., L.T. and Hydr. L.T.) are due largely to differences in the toxicity of the dimethyl sulphate-soluble fractions.

ROLE OF TAR PHENOLS AND TAR BASES.

There is little information available on the part played by these constituents although Martin (1935) quoted work which suggested that tar phenols might not be very toxic and that their presence might be deleterious owing to a diluent effect. Shaw and Steer (1947b) found that both phenols and bases were of low toxicity to the eggs of the Winter Moth, *Operophtera brumata* L., although in the oils examined they were not present in sufficient quantity to dilute more active constituents. In the present work with *A. pomi* eggs, the question was studied for an L.T. oil distilling above 250° C. The characteristics of this oil and the neutral oil derived from it were as follows :

Oil.	Neutral oil % by weight.	Sp. gr.	Viscosity Redwood I at 70° F.	Insol. in Me_2SO_4 %
L.T. whole ..	79·1	1·040	—	29
L.T. neutral ..	100·0	1·021	99	26

The neutral oil, phenols and bases obtained from the L.T. whole oil were emulsified separately with 1·0 per cent. sodium oleate solution and tested biologically, with results shown in Table XVI.

The probit-log. dosage regression lines derived from these data are :—

(i) L.T. whole : $Y = 2{\cdot}24x + 1{\cdot}84$ LD50 = 2·55 per cent.
(ii) L.T. neutral : $Y = 4{\cdot}51x - 1{\cdot}12$ LD50 = 2·27 per cent.
(iii) L.T. phenols : $Y = 4{\cdot}69x + 0{\cdot}30$ LD50 = 1·02 per cent.
(iv) L.T. bases : $Y = 6{\cdot}67x + 0{\cdot}52$ LD50 = 0·47 per cent.

A comparison of L.T. whole with the neutral oil showed that the two lines differ significantly in slope so that no evaluation of relative potency is possible. Comparison of the phenols and bases with the neutral oil is permissible in terms of relative potencies and reveals them to be respectively 2·2 ± 0·16 and 5·2 ± 0·30 times as toxic as the neutral oil, both differences being highly significant. The peculiar feature of these results is that, if toxic effects were additive, one would expect the neutral oil to be somewhat less toxic than the whole oil. In fact, the latter gives a lower percentage kill over the whole range of concentrations at which the two were tested and this suggests very strongly that there is antagonism (negative synergism) between the neutral oil and the phenols and/or bases. It is, however, a complicated situation as the probit-log. dosage regression line for the mixture is not parallel to those for its components and, in view of the unsatisfactory state of the statistical theory of synergism (except in the simple case of similar-action synergism with a common regression coefficient for the mixture and its components ; Finney, 1947), no attempt has been made to analyse the relationships involved.

TABLE XVI.

Toxicity of L.T. whole, neutral, phenols and bases.

Test No.	Date of treatment.	Oil.	Conc. %	Mean % hatch.	S.E.	Total number of eggs.	Test No.	Date of treatment.	Oil.	Conc. %	Mean % hatch.	S.E.	Total number of eggs.
13/38	21.2.39	L.T. whole > 250° C.	5·0	20·5	5·67	1,727	13/38	21.2.39	L.T. phenols > 250° C.	1·0	40·6	3·62	1,554
			4·0	25·6	2·04	1,347				0·7	67·8	5·17	1,351
			3·0	42·3	7·65	1,249				0·45	75·1	4·26	1,104
			2·0	46·2	2·43	1,035				0·3	86·5	3·24	1,322
			1·0	68·0	4·40	1,220							
14/38	21.2.39	L.T. neutral > 250° C.	6·0	1·9	0·96	1,554	13/38	21.2.39	L.T. bases > 250° C.	1·0	0·0	—	1,508
			4·5	9·9	2·31	1,430				0·7	8·0	2·55	1,353
			3·5	15·8	3·46	1,480				0·45	51·6	3·39	1,711
			2·5	33·7	1·73	1,800				0·3	73·0	1·66	1,285
			1·5	67·2	3·82	1,590				0·2	78·4	4·82	1,190

Control: 83·2 ± 5·48 (4,389).

TABLE XVII.

Toxicity of H.T. neutral oil in presence of other oils.
Test No. 11-12/38. Date of treatment : 20.2.39

Conc. H.T. neut. > 250° C. %	+ 20·0% spindle.			+ 40·0% spindle.			+ 40·0% paraffin 200-250° C.			+ 40·0% H.T. neutral 200-250° C.		
	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.
1·5	0·4	0·18	1,185	0·4	0·35	1,529	0·1	0·06	1,452	0·1	0·07	1,739
1·0	1·5	0·34	1,537	0·6	0·19	1,428	0·4	0·20	1,539	0·2	0·32	1,232
0·75	9·7	1·59	1,332	2·7	1·57	1,319	10·4	0·68	1,185	5·5	3·30	1,047
0·5	12·9	4·30	1,405	21·8	2·44	1,549	20·8	5·29	1,593	24·2	2·81	1,774
0·25	37·0	4·04	1,287	46·4	3·92	1,661	37·8	2·55	1,061	58·8	4·75	1,689

Controls : 11/38 : 92·1 ± 2·72 (1,879).
12/38 : 91·8 ± 1·33 (4,857).

JOINT ACTION OF HYDROCARBON OILS.

Current practice in the formulation of tar oil winter washes involves the admixture of either paraffin or a spindle oil with the tar distillate, primarily to facilitate the manufacturing process. This fact suggested the desirability of examining mixtures of toxic and relatively non-toxic hydrocarbon oils, and tests were made to determine :—

(i) The extent to which the toxicity of H.T. neutral oil is altered by the addition of other, virtually non-toxic oils.

(ii) The mode of joint action of H.T. and L.T. neutral oils.

Tables XVII and XVIII summarize the biological data obtained in these tests. In the former, the percentage of added oil is in terms of the concentration of H.T. neutral oil > 250° C.

Table XVIII.

Toxicity of H.T. neutral and L.T. neutral mixtures.

Test No.	Date of treatment.	Mixture.	Total oil conc. %	Mean % hatch.	S.E.	Total number of eggs.
11/38	20.2.39	H.T. > 250° C.	1·5	0·0	—	1,939
			1·0	2·6	1·53	1,343
			0·75	5·8	0·63	2,138
			0·5	14·9	2·77	1,884
			0·35	28·3	7·42	2,223
			0·25	27·1	7·98	2,282
14/38	21.2.39	70% H.T. > 250° C. plus 30% L.T. > 250° C.	1·6	2·1	1·23	1,536
			1·2	10·0	1·00	1,078
			0·8	36·1	6·05	1,717
			0·6	30·4	8·18	1,142
			0·45	53·4	2·73	1,187
14/38	21.2.39	30% H.T. > 250° C. plus 70% L.T. > 250° C.	2·8	2·3	0·41	1,363
			1·8	21·5	2·44	1,503
			1·35	33·3	0·66	1,438
			1·0	70·5	2·74	1,247
			0·8	73·1	4·65	626*
14/38	21.2.39	L.T. > 250° C.	6·0	1·9	0·96	1,554
			4·5	9·9	2·31	1,430
			3·5	15·8	3·46	1,480
			2·5	33·7	1·73	1,800
			1·5	67·2	3·82	1,590

* 4 replicates. Controls : 14/38 : 83·2 ± 5·48 (4,389).

Considering first the effect of adding various other oils to H.T. neutral oil, the following probit-log. dosage regression lines were calculated from the data of Table XVII :

(i) H.T. Neutral > 250°C. $Y = 2{\cdot}47x + 4{\cdot}36$
(ii) ,, plus 20 per cent. Spin. $Y = 2{\cdot}72x + 4{\cdot}13$
(iii) ,, plus 40 per cent. Spin. $Y = 3{\cdot}49x + 3{\cdot}51$
(iv) ,, plus 40 per cent. Paraffin. $Y = 2{\cdot}93x + 3{\cdot}89$
(v) ,, plus 40 per cent. H.T. neutral (200-250° C.) $Y = 4{\cdot}07x + 2{\cdot}94$

On comparison, lines (ii), (iii) and (iv) were found not to differ significantly in slope or position from (i) and it may, therefore, be concluded that, within the limits of experimental error, these oils do not affect the toxicity of H.T. neutral oil in mixtures. On the other hand, the addition of the low-boiling H.T. fraction results in a regression line, (v), which differs both in slope and position from the standard, the two lines intersecting at a probit value corresponding to about 95 per cent. mortality. The details of this interaction could not be further pursued as no toxicity data are available for the low-boiling H.T. fraction, but it is probable that the concentration of H.T. neutral oil required to achieve control of *A. pomi* would not be greatly affected in the mixture.

In the second case, the following probit-log. dosage regression lines for H.T./L.T. mixtures were derived from the data of Table XVIII :

(i) H.T. neutral $Y = 2{\cdot}47x + 4{\cdot}36$
(ii) 70% H.T. + 30% L.T. $Y = 3{\cdot}65x + 2{\cdot}20$
(iii) 30% H.T. + 70% L.T. $Y = 5{\cdot}89x - 1{\cdot}63$
(iv) L.T. neutral $Y = 4{\cdot}45x - 0{\cdot}95$

These lines differ significantly from parallelism ($P < 0{\cdot}01$ from a joint test of significance carried out by partitioning the total χ^2 for all observations into one component representing parallelism and another associated with residual heterogeneity and then carrying out a variance-ratio test using the χ^2 for residual heterogeneity as the error mean square). This being the case, the possibility of similar joint action is immediately ruled out, but the limitations of existing methods of detecting and assessing synergism when the regression coefficients of the mixtures and their components differ (Finney, 1947) makes further attempts to analyse the situation unprofitable beyond pointing out that in the neighbourhood of a complete kill the toxicity of H.T. neutral is not greatly changed by admixture with L.T. neutral.

DATE OF APPLICATION OF OVICIDES IN RELATION TO TOXICITY.

The extent to which changes in the susceptibility of the eggs to ovicidal washes occur during the course of the season was studied by treating eggs on six occasions at three different concentrations of H.T. neutral oil. The specification of the oil used is shown below and the results of the biological tests are given in Table XIX.

Neutral oil, per cent. by weight	97·4
Specific gravity	1·084
Insol. in Me_2SO_4, per cent.	0·0
Unsulphonated residue, per cent.	0·0
Distillation range : 10 per cent. distils at 242° C.	
50 ,, ,, ,, ,, 306° C.	
80 ,, ,, ,, ,, 354° C.	

These data are not very suitable for analysis by means of a Bliss transformation to probits and logarithms of oil concentrations, but comparisons of the percentage hatches at 2·0 per cent. by means of Pitman's t-test applied to pairs of times shows that there is no significant effect of occasion at this concentration. Analyses of variance carried out on the remaining data showed some significant effects of time of

TABLE XIX.

Effect of time of application on toxicity of H.T. neutral oil.

Date of treatment.	Concentration of oil.									Controls.		
	2·0%			1·0%			0·5%					
	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.	Mean % hatch.	S.E.	Total number of eggs.
20.1.44	5·0	4·3	2,942	31·8	1·3	1,743	73·8	4·0	1,358	70·8	9·5	2,422
4.2.44	1·0	0·4	1,040	20·5	2·7	1,512	70·7	3·7	2,084	81·7	2·2	2,855
17.2.44	8·1	3·6	2,415	18·4	8·6	2,386	50·0*	8·8	1,735	80·4	1·6	1,692
4.3.44	0·0	—	2,385	7·9	2·2	1,459	33·3	4·0	2,206	63·9	7·7	1,349
10.3.44	0·4	0·2	2,287	7·8	4·5	2,385	43·8	2·9	3,282	71·7	5·3	2,069
17.3.44	0·1	0·1	2,390	5·2	2·4	2,435	5·1	3·7	1,587	71·6	4·2	1,446

Bracketed values do not differ significantly.
* Does not differ significantly from hatch of 43·8% on 10.3.44.

application (bracketed data in Table XIX do not differ significantly) and justify the conclusion that the effects of later applications depend largely on the increased susceptibility of the eggs to the lower concentrations of the range employed. This would seem to mean that the only practical advantage of using a tar oil wash late in the dormant season is the increased likelihood of killing eggs which receive less than the average amount of wash.

GENERAL DISCUSSION.

The clearest conclusion emerging from the experimental work described above is that no single characteristic of the oils investigated can be correlated with toxicity—a result similar to that obtained in Part II of this series with eggs of the Winter Moth, *Operophtera brumata* L. On the other hand, within each oil type there is abundant confirmation of the association of toxicity with distillation range noted by earlier workers and the importance attached to this property in current tar-oil wash specifications is justified. Distillation range alone, however, is clearly not a sound basis for assuming that the oil is necessarily of high toxicity.

In the case of lepidopterous eggs, Pearce, Chapman and Avens (1942) and Shaw and Steer (1947b) found the various components of hydrocarbon oils to increase in toxicity in the order aromatics, naphthenes, paraffins. It is obvious that towards eggs of *A. pomi* the paraffinic oils (Spin.) are of very low toxicity, while a wholly aromatic oil like H.T. neutral is highly toxic. The role of naphthenic components cannot be directly assessed from the present experiments but, since the dimethyl sulphate-insoluble fractions of V.T. (mainly naphthenic) and L.T. (largely paraffinic) do not differ significantly in ovicidal power, it seems legitimate to conclude that the naphthenes are also of very low toxicity. Only the dimethyl sulphate-soluble fractions gave indisputable evidence of contributing towards the toxicity of the neutral oils and this suggests that any further work on the ovicidal properties of tar distillates and similar oils should be directed towards a more precise characterization of the aromatic components.

While it appears to be broadly true that a high percentage of oil soluble in dimethyl sulphate, together with a satisfactory distillation range, is correlated with high toxicity, and that the converse also holds, this generalization requires important qualifications. The experiments on narrow fractions show that the order of toxicity of different oil types of similar distillation range is not the same as the order of their solubility in dimethyl sulphate. Obviously connected with this is the further observation that isolated dimethyl sulphate-soluble fractions from the different types of distillate (cuts distilling > 250° C.) differ considerably in their toxicity.

The conclusion is inescapable that there exist several groups of aromatic compounds differing widely in their toxicity to eggs of *A. pomi* in a way that cannot be explained by differences in distillation range. The most toxic are those compounds of comparatively simple structure obtained from high temperature carbonization coal tars, i.e. H.T. and V.T. oils. Considerably less toxic are the more complex compounds derived from low-temperature carbonization tars and those aromatic compounds, e.g. the Edeleanu extract, found in petroleum oils. This may indicate that while these heavily substituted molecules retain sufficient of their aromatic character to be classed as aromatic by the criterion of solubility in dimethyl sulphate the influence of their aliphatic substituents is such as to reduce appreciably their toxicity to *A. pomi* eggs. Thus, solubility in dimethyl sulphate can be regarded as a satisfactory indication of toxicity only when applied to high-temperature carbonization products. Outside this group, e.g. with low-temperature tar distillates, it can be seriously misleading.

Some of the results of this present investigation as well as earlier work find expression in the current official specifications for tar oil winter washes (Ministry of Agriculture, 1941). These lay down the required amount and distillation range of the neutral oil soluble in dimethyl sulphate and specifically exclude all products other than distillates from high-temperature carbonization tars.

The isolated phenols and tar bases possess greater toxicity than do the neutral oils, but in the case of L.T. oils there is evidence of antagonism between these constituents and the neutral oil. This observation emphasizes the need for an investigation of the mode of joint action of the pure compounds, or groups of such compounds, that occur in tar oils, e.g. tar bases in the presence of paraffins, tar phenols in the presence of naphthenes, etc.

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SUMMARY.

A survey of the ovicidal properties of a selection of tar and petroleum oils of varied physical and chemical characteristics was conducted, using the eggs of *Aphis pomi* Deg. as a test organism. Evidence is presented to show that toxicity is determined mainly by the aromatic constituents of the oils, naphthenic and paraffinic fractions being of low toxicity. Toxicity cannot be correlated with any single property of the various oils although distillation range and solubility in dimethyl sulphate jointly afford some guide to biological performance. This latter correlation, however, is far from complete since aromatic fractions from different oil types, all distilling above 250° C., differ considerably in their toxicity. Tar phenols and bases separated from a low temperature tar distillate are more toxic than is the remaining neutral oil, but there is some evidence of antagonism between the neutral oil and the phenols and/or bases. The susceptibility of the eggs to a horizontal retort high-temperature tar distillate increases late in the season.

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NUT ROTTING IN *CORYLUS AVELLANA* L. IN RELATION TO THE ACTIVITIES OF THE NUT WEEVIL, *BALANINUS NUCUM* L.*

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In 1943, attention was drawn to a troublesome disease of cobnuts and filberts in commercial plantations. Wormald (1944) described the symptoms and stated that the disease appeared to be unrecorded previously in Britain, though in the light of the investigations described below it seems very likely that it had existed unnoticed for some time before 1943. Affected nuts, which show brown discoloration of the cupules, the shells, and the tissue† within the shells, drop freely in an immature condition in July, and heavy loss of crop may result.

The conidial stage (*Monilia fructigena* (Fr.) Westend.) of the Brown Rot fungus (*Sclerotinia fructigena* Aderh. and Ruhl.) was commonly associated with the rot symptoms, and was suspected as the chief cause by Wormald (1944), who suggested the common name " Nut Drop " for the disease.

LITERATURE.

In Germany, Sorauer (1900) had found and described a similar disease in 1887 in the botanic gardens of the Pomological Institute at Proskau, and had shown by inoculation experiments that hazel-nuts, peaches, apricots, and grapes could be infected with a *Monilia* (species not stated) obtained from apple, and that apples, plums, rose-hips, and grapes could likewise be infected from hazel-nuts. He alluded to the presence on the immature nuts of numerous wounds often invested with a black mould (*Cladosporium* sp.) as an accompanying feature of the disease.

Schellenberg (1906) described a disease of hazel-nut found widely in Switzerland, and attributed it to a *Sclerotinia*, for which he proposed the combination *S. Coryli* Schellenb. Apothecia found on the male catkins were described, and a connexion between this phase and *Monilia* on rotting fruits was postulated but not proved by experiment.

Wahl (1924) mentioned that *Monilia fructigena* caused appreciable losses on hazel-nuts in Carinthia, but offered no reason why the fungus was referred to that species.

Eriksson (1930, p. 401) described " A Sclerotinia disease of the Hazel Nut (*Corylus Avellana*) ", which is attributed to the allied fungus *S. cinerea* (now *S. laxa*), the cause of Blossom Wilt and Fruit Rot chiefly of stone fruits. Both Sorauer and Eriksson mention the possibility of infection through the stigma, but it seems probable from this and other references that Eriksson was largely quoting Sorauer's views.

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* Part of a Thesis approved for the Degree of Doctor of Philosophy in the University of London.

† The tissue that fills the shell at this stage is white and pithlike in appearance. It disintegrates as the kernel (seed) progressively enlarges, and the remains are loosely attached to the skin (testa) of the ripe kernel and to the inner walls of the shell, usually in brown strands. For the purpose of this paper the term " inner tissue " will be used in reference to it.

Stigma-infection is usual with *S. laxa* but not with *S. fructigena*, although this fungus can readily infect flowers, as demonstrated by Woronin (1900) in inoculation experiments on apple. *S. fructigena* does not produce conidial pustules until much later in the year under normal field conditions and, by then, hazel stigmas would be dry and withered and the nutlets developing. Reference to an original paper by Eriksson (1913) showed that he in fact made no distinction between *M. cinerea* (*S. laxa*) and *M. fructigena* because of his view that a species diagnosis from the colour of the conidial cushions is always doubtful. This lack of distinction is further evident in Eriksson's recommendations for the control of the nut disease, which are the same as those he advanced for Brown Rot of apples (*S. fructigena*). In Britain the measures advised for controlling *S. fructigena* on apple (Wormald and Moore, 1945) are mostly inappropriate for *S. laxa*. Though the removal of sources of infection is obviously important for either fungus, the more direct control measures lay the emphasis, with *S. fructigena*, on the prevention of wounds in the fruit and, with *S. laxa*, on the application of a caustic spray during the dormant season to destroy winter conidial pustules that arise on spurs and laterals killed by the fungus, an application useless for *S. fructigena*.

In the original German version of his book, Eriksson (1928) stated that the disease caused considerable losses on Cosford's Seedling and on wild hazel in South Sweden in 1911-12.

Johansen (1937) working in Denmark, tied Myrobalan fruits infected with *M. fructigena* to unwounded apples and hazel-nuts *in situ*, and was able to transmit the infection in either case, though only two out of twenty-five nuts became infected under these conditions. Her conidial measurements showed agreement with those for *M. fructigena*.

Buchwald (1943), also working in Denmark, undertook an investigation on hazel-nuts to settle the divergences of opinion on the specific identity of the causal fungus. His inoculation experiments, cultural studies, and conidial measurements showed conclusively that the disease was caused by *S. fructigena*. In commenting on previous work, Buchwald stated that he could not comprehend Eriksson's reference of the causal fungus to *S. cinerea*, especially as it followed the note by Wahl (1924), who appears to have been the first to refer the fungus to (*S.*) *M. fructigena*. Eriksson's reference was apparently followed by Ferraris (1938). Buchwald further considered that the suggestion of Schellenberg (1906) to attribute his hazel-nut disease to *S. Coryli* cannot be accepted, and he proposed that the name *Ciboria Coryli* (Schellenberg) *n. comb.* should be substituted for it.

Miller (1945), in a survey of diseases of the filbert in Oregon and Washington, does not mention this rot disease.

A. SPRAYING EXPERIMENTS, 1944-46.

The grower who first reported the disease had in 1944 already begun measures for its control by spraying, and as nut bushes were not available for experiments on the Research Station farm, it was decided to make observations on this preliminary trial and to act in an advisory capacity to the grower in his efforts to improve his crop. In 1945 the trial was replanned by the writer, and full facilities were accorded by the grower for observation and making records.

Results in 1945 showed that the problem of crop loss was not being satisfactorily solved by the spray treatments and that the incidence of the disease needed further study as a basis for future treatment. Meantime, the spraying trial was further modified for 1946 primarily to afford the grower maximum benefit from the meagre information already accrued, and the area of land occupied by a control treatment was therefore reduced in the interests of total crop.

An account of these early spraying experiments follows.

EXPERIMENT I, 1944.

Lime-sulphur (2 per cent.) with lead arsenate (0·4 per cent.) (L.S.A.) was compared with hydrated-lime Bordeaux mixture (8-8-100) (B.M.) and with copper-lime dust (C.L.), and untreated rows were retained as controls. Two applications were made, on 11th and 26th May. The Bordeaux caused considerable leaf-scorch, but the only data obtained were of total crop per treatment, the figures being supplied by the grower.

The crop was greatly enhanced by spray treatment, and was improved a little by dusting, there being practically no crop on the untreated bushes.

L.S.A.	B.M.	C.L. (dust)	CONTROL
11·0	11·0	3·3	0·6

The figures represent an approximate average crop in half-sieves (20 lb.) per row, averaging some 70 old bushes (i.e. about 3 lb. per bush when sprayed) and served merely as a starting-point for future work ; there was no evidence to show how the increase in crop could be explained.

EXPERIMENT II, 1945.

Method.

The same bushes (planted in 30 rows, in all comprising over 2,000 bushes) were included in a replanned trial in which six treatments were compared, five rows to each. They were :

1. Colloidal copper preparation diluted to contain 0·05 per cent. Cu.
2. As 1, with the addition of lead arsenate powder (0·2 per cent.).
3. Lead arsenate powder (0·2 per cent.) alone.
4. Lime-sulphur (2 per cent.).
5. As 4, with the addition of lead arsenate powder (0·2 per cent.).
6. Untreated controls.

Treatments were applied twice, on 4th and 16th May, by means of a mobile sprayer supplying adequate spray through three-nozzle " brooms " at 400-500 lb. pressure per sq. in. A link with the grower's experiment was provided by the use of 2 per cent. lime-sulphur with and without lead arsenate ; colloidal copper was preferred to Bordeaux mixture because it is more easily prepared, and it was diluted to about one-quarter of the copper content of the Bordeaux, which had proved much too phytotoxic. Lead arsenate was included as the standard recommendation for Nut Weevil control so that any crop loss from this pest could be determined separately from that caused by the Brown Rot fungus.

Records.

The rotting nuts started dropping in mid-July and, on the 25th, a record was made of the total of dropped nuts on one side of every tenth bush in the three middle rows of each treatment. The crop, a poor one, was harvested and recorded by the grower in autumn, and a random sample of 400 nuts from each treatment was examined for circular holes in the shell—evidence of the escape of the mature Nut Weevil larvae.

Results.

Foliage injury resulting from the sprays was negligible. The crop data, summarized in Table I, show that lead arsenate reduced dropping in July and gave some control of Nut Weevil on the harvested crop, the fungicides alone having no effect in either respect. The beneficial effect of arsenate was reflected also in the total crop harvested, though this was meagre at the best, and the presence of either fungicide with the arsenate contributed.

TABLE I.

Results in 1945.

Treatment.	Av. dropped nuts per bush in July.	lb. crop/ no. bushes.	lb. per bush.	Weevil at harvest (sample 400 nuts).
1. Colloidal Copper ..	31	80/202	0·4	21
2. Same + Arsenate ..	15	283/240	1·2	11
3. Arsenate alone	16	129/235	0·5	8
4. Lime-sulphur	36	32/235	0·1	40
5. Same + Arsenate ..	16	230/200	1·2	7
6. Untreated controls ..	21	91/209	0·4	23

The failure of the fungicides by themselves to sustain the promise indicated in 1944 suggests that where lime-sulphur was used in that year, the increase in crop weight might have been chiefly due to the insecticidal value of the lead arsenate component and, where Bordeaux was used, to some undetermined insecticidal or fungicidal action of this spray when applied too strong for safety. Whatever the explanation, however, it is clear from the 1945 (July) data that the chief crop loss was related to the absence of lead arsenate in the spray. Nut Weevil, which was only imperfectly controlled by the lead arsenate sprays applied, thus appeared to be a primary agent in loss of crop. The part played by the Brown Rot fungus was uncertain ; it was clearly important in the plantation, but the incidence of the disease was apparently unaffected by the application of fungicides alone. More information was needed on the method by which immature nuts were induced to drop. Meantime, Experiment III was planned chiefly in the grower's interests.

EXPERIMENT III, 1946.

Method.

The same 30 rows were divided into three unequal strips and treated as follows :

Rows 1-12. Colloidal copper (C.C.) preparation diluted to contain 0·05 per cent. Cu. + lead arsenate powder (A) at 0·2 per cent.

Rows 13-17. Lead arsenate powder (0·2 per cent.) as control.

Rows 18-30. Lime-sulphur (L.S.) at 2 per cent. + lead arsenate powder (0·2 per cent.).

Two applications were made, at the middle and end of May, nicotine being included for aphid control ; the same mobile sprayer was used as hitherto. A third application corresponding to the others was made in the third week of June on one-half only of certain rows in each treatment so that two applications of each could be compared with three.

Records.

These were made in mid-July, when kernels were beginning to enlarge in the most forward nuts and infected nuts and clusters had just started to drop. Numerous adult Nut Weevils were found on the bushes, together with freshly deposited and some recently hatched eggs (identified by Dr. A. M. Massee as those of the Nut Weevil, *Balaninus nucum* L.) in the nuts. Laterals bearing only one nut were chosen to avoid the observed tendency for infection to travel across the cluster base from a primarily infected nut to another in the same cluster. Single nuts were examined at random on each side (150 nuts) of each of six rows, representing all treatments.

Results.

The numbers of rotting nuts out of 300 per treatment are set out in Table II; they indicate an inferiority of colloidal copper + arsenate to lime-sulphur + arsenate, which showed slight improvement on arsenate alone where three sprays were given. The third application had a marked beneficial effect.

TABLE II.

Results in 1946.

	No. rotting in sample of 300 nuts.	
Treatment.	Two sprays.	Three sprays.
C.C.A.	42	31
A.	30	18
L.S.A.	26	11
Mean	33	20

Figures for final crop are not available; the grower wrote on 16th October, 1946: " I am afraid it is again a very small crop."

DISCUSSION OF EVIDENCE FROM THE SPRAYING EXPERIMENTS.

The fungicides had evidently had little controlling influence on rotting except where a harmful Bordeaux mixture was tested in 1944; indeed, colloidal copper apparently increased it in 1946, though positional effect cannot be ruled out in the absence of replication. For the same reason the apparent beneficial effect of lime-sulphur may be due to chance.

Dropping in July was reduced only by the presence of lead arsenate in the spray of 1945, and in 1946 a later (June) application was clearly beneficial against rotting. In short, the insecticidal element in the spraying had proved more important than the fungicidal, and the later the spray was applied before the dropping began, the better the result. Yet nuts probed by Nut Weevil do not invariably drop; many infested ones can be found in the crop picked at harvest, as shown by the 1945 data. Some connexion between the oviposition probings and the incidence of the rotting (this then causing affected nuts to drop) was clearly likely, for most of the dropping closely followed the ovipositing activity of the Weevil. Extended examination early in July, 1946, had in fact shown that almost every infected nut bore an oviposition tunnel (Fig. 1)* often containing a freshly-deposited egg ; some of the nuts were beginning to rot around the site of the tunnel (Moore, 1947). A few days after this the rotting nuts started to drop, including whole clusters even where only one nut in the cluster was rotting, as mentioned by Sorauer (1900).

These observations provided strong support for the tentative conclusion from the spraying trials, though they ran counter to existing information on the habits of the Nut Weevil. Massee (1946, p. 146), for instance, states that " The grub hatches in June and feeds on the kernel . . .". Moreover, the standard spray programme for this pest involves the use of lead arsenate in May and early June, though a spray during the second half of June had in the present experiments shown marked promise in reducing rotting.

The time was now opportune for more detailed study of the incidence of the disease.

B. INOCULATION EXPERIMENTS, 1946-48.

I. To investigate the incidence of rotting and premature dropping of nuts in the plantation, and the protective effect of a fungicide, 1946.

Method.

Inoculations were made on 11th July on 80 selected clusters of growing nuts as follows :

Clusters.	Needle-punctured.	Inoculated.
20	+	+
20	+	–
20	–	+
20	–	–

Each series of clusters was sub-divided into 10 copper-sprayed and 10 not copper-sprayed, giving 8 series of 10 similarly-treated clusters.

Spraying. A preparation of copper oxychloride, diluted to contain 0·05 per cent. Cu, was applied to each nut in the appropriate cluster from a hand atomizer of the scent-spray type. The deposit was allowed to dry before any further treatment was given.

Inoculating. A conidial suspension containing approximately 1,000 spores per ml. was prepared in distilled water from fresh pustules of *Monilia fructigena* on Czar plum fruits growing on interplanted trees in the nut plantation. Each nut in every appropriate cluster was sprayed with this suspension from a hand atomizer, an average of 800 spores being applied per nut.

Puncturing. A flamed steel needle was pushed right through each appropriate nut just above the clasping base of the cupule ; the nuts were still soft-shelled.

The operations were carried out in the order stated, and the clusters were labelled and left unprotected.

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* It seems probable that the " feed-passages " (" Frassgänge ") mentioned by Sorauer (1900) as being abundant on many of the nuts he examined were in fact oviposition sites of the Nut Weevil similar to those recorded here.

Results.

These were apparent by 18th July, one week after inoculation. Clusters containing rotting nuts, some bearing spore-cushions of *M. fructigena*, were just about to separate from the parent lateral. All the nuts were collected and classified, and the following data (Table III) were obtained.

TABLE III.

Results of Inoculations, 1946.

	Needle-punctured.	Inoculated.	Copper Sprayed.		Not Sprayed.	
			Total nuts.	Rotting nuts.	Total nuts.	Rotting nuts.
A	+	+	23	20	25	23
B	+	–	26	26	22	20
C	–	+	26	9*	31	3*
D	–	–	25	5*	24	4*
		Total	100	60	102	50

* Each was found to be probed by Nut Weevil.

The copper spray did not prevent rotting, and the addition of the spore-suspension did not increase it: evidently there was enough inoculum already from rotting apple and plum fruits overhanging the nuts. The only effective treatment was puncturing with a needle, and the problem, one of wound parasitism, was thus seen to bear a close parallel to that of Brown Rot of apple. Nearly all of the rotting nuts ultimately bore spore-cushions of *M. fructigena*. Not all punctured nuts rotted, but the presence of Weevil probings was shown by the experiment itself to be enough to account for the rotting of 21 nuts (Table III) that had not been needle-punctured.

The pathogen for each of the 26 rotted nuts in series B (copper sprayed) was recovered in James Grieve apples under sterile conditions in the laboratory. The procedure was described in an earlier report (Moore, 1947); 24 apples developed Brown Rot (*M. fructigena*), one developed Grey Mould (*Botrytis cinerea*), and one remained uninfected, as did all the controls. The results are illustrated in Fig. 2.

Apparently *Monilia fructigena*, though the chief, was not the only cause of the rotting. As few special precautions were taken for the inoculations in the plantation, there was opportunity for any available wound parasite to effect entry. In view of the occurrence of *Botrytis cinerea* in this instance, a further experiment was undertaken to test its pathogenicity on nuts under sterile conditions. At this period in late July, however, it was found that the shells of the nuts were rapidly hardening so that it was difficult to puncture them with a needle. Moreover, the predations of the very numerous Nut Weevil population had rendered it difficult to find many still soft-shelled yet unprobed nuts. A further complication was the discovery that certain nuts from a sample earlier retained for this experiment showed brown inner tissue when cut across, although there was no external sign of infection. Hence, to ensure that only sound nuts with white inner tissue were used, the choice was limited, and five fresh nuts cut longitudinally in half were therefore taken as the experimental unit.

II. To confirm the pathogenicity of *Botrytis cinerea* on nut, 1946.

Method.

Small portions of the rotting flesh of the appropriate apple from Experiment I were planted under sterile conditions in the middle of the inner tissue of each of the ten half-nuts, which were then incubated in sterile petri-dishes. A similar series remained uninoculated as control.

Results.

On the third day after inoculation the controls were still sterile ; the nuts inoculated with *Botrytis* were rapidly rotting, and the cut surfaces and shells were covered with vigorous, dense, fertile growth typical of this fungus under favourable conditions. The *Botrytis* was evidently an active wound parasite of cobnuts under these conditions.

Other experiments were held over until 1947 in view of the difficulty of obtaining further suitable material.

III. To confirm the pathogenicity of *Monilia fructigena* on nut, 1947.

Method.

On 15th July, a sample of unprobed, healthy nuts was collected from the plantation. Of these, 100 were selected for the inoculation experiment and were stripped of the loose parts of the cupule ; they were surface-sterilized with absolute alcohol and were pierced with a sterile steel needle right through the nut just above the clasping base of the cupule, thus making two holes in each nut. Two series of 50 nuts each were put into large sterile petri-dishes with no added moisture.

A dense conidial suspension of *Monilia fructigena* was prepared in sterile distilled water from spore-cushions growing on a fresh lesion on a surface-sterilized Allington Pippin apple fruit. One series of nuts was inoculated by transferring a loopful of spore-suspension to one of the two needle-holes in each nut ; the other series received only sterile distilled water as control. This method of inoculation proved highly satisfactory, as the loopful of liquid was immediately sucked into the hole, thus depositing spores in the inner tissue.

Results.

Three days later the nuts in the inoculated series were becoming discoloured, and several were already showing typical spore-cushions of *M. fructigena*. Some of the nuts in the control series, though uninfected, were becoming patchily brown, probably owing to the alcohol (Fig. 3). By 21st July the inoculated nuts were mostly covered with a mass of fungal growth ; the others were still apparently sterile.

The pathogen was re-isolated in a pure state, with surface-sterilized apple fruits as medium, from each nut of a random sample of 24 inoculated ones. Similar transfers made from uninoculated nuts, nearly all of which had brown inner tissue, gave negative results. The shells of the inoculated nuts, but not those of the uninoculated ones, were found to crush readily through disintegration resulting from infection.

By this time most of the inoculated nuts had developed spore-cushions of *M. fructigena* ; they develop fairly readily on soft-shelled nuts but only sparsely when the shells are hardening, usually during the second half of July.

It had thus been clearly confirmed that *M. fructigena* is a ready wound parasite of hazel nuts in Britain as in Denmark (Buchwald, 1943). Its usual mode of entry is evidently through the oviposition punctures of the Nut Weevil; this would elucidate the difficulty found by Johansen (1937) in infecting unwounded nuts.

This experiment had also confirmed that uninfected nuts may contain brown inner tissue, which could be misleading in diagnosis, though possibly not a serious complication as there are other symptoms of fungal invasion. Later experiments showed the browning to be a symptom of wilting, which did not occur when nuts were kept in moist air after picking.

IV. TO TEST THE WOUND PARASITISM OF CERTAIN OTHER FUNGI ASSOCIATED WITH ROTTING IN NUT, 1948.

In the course of the examination of numerous specimens of unhealthy nuts, certain fungi other than *M. fructigena* and *B. cinerea* were sometimes encountered, e.g. species of *Fusarium*, *Cladosporium*,* *Alternaria*, and *Cytospora*.

Method.

Cladosporium herbarum and a species of *Fusarium* bearing pink sporodochia, each encountered on several occasions, were isolated in pure culture in July. A sample of sound, healthy nuts was collected and 80 were selected for inoculation. They were stripped of loose cupule, surface sterilized with alcohol, and divided into four series of 20 nuts each, treated as follows:

(*a*) Not inoculated.
(*b*) Inoculated with *Fusarium* sp.
(*c*) ,, ,, *Cladosporium herbarum.*
(*d*) ,, ,, *Monilia fructigena*, isolated from a rotting nut, and included as a basis for comparison.

A small portion of the top of each nut was removed with a sterile scalpel, which was then inserted vertically in the suture and twisted sideways so that the nut was cracked partly open. A portion of the appropriate agar culture was inserted, and the four series of nuts were stored separately in large, sterile petri-dishes without added moisture.

Results.

A week later none of the control nuts had rotted, but most of those inoculated with *M. fructigena* showed advanced rot symptoms with vigorous aerial mycelium. Six bore spore-cushions of the fungus. A few were apparently rotting but slowly; an accurate count of these was rendered difficult by discoloration presumably due to wilting. All the nuts inoculated with *Fusarium* sp. were well rotted and bore copious pinkish aerial mycelium; the fungus was actively parasitic under these conditions. By contrast, the nuts inoculated with *C. herbarum*, though mostly discoloured, showed only very localized evidence of rotting. In some, growth had apparently progressed a little from the point of inoculation, but again it was difficult to be certain how much of this was mere discoloration.

A portion of the inner tissue of each nut was extracted from the basal part of the nut, i.e. the end opposite to the inoculated one, and transferred to prune-agar plates. Of the controls, twelve plantings were sterile, and eight gave rise to fungal growth, including species of *Alternaria* and *Cephalosporium*, presumably chance contaminants that had arisen during examination of the nuts after incubation.

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* See also Sorauer (1900).

Monilia fructigena and the *Fusarium* sp. were readily recovered in a pure state from all appropriate plantings, but with *C. herbarum* ten plantings gave rise to the original fungus and the other ten, like the controls, to chance contaminants including *Alternaria* sp.

Conidial measurements of the *Fusarium* sp. on malt agar ranged from 22-38$\times$3-4μ (mean 30·3$\times$3·7μ). The spores were mostly slightly curved, hooked at one end, and three-septate. Lacey (1948) reported that the fungus produced the antibiotic " lateritiin ", and W. L. Gordon, Dominion Rust Research Laboratory, Winnipeg, identified the pathogen as *F. lateritium sensu* Snyder and Hansen.

Discussion of evidence from the inoculation experiments.

It may be concluded that *Fusarium lateritium* is, like *Botrytis cinerea*, an active though minor wound parasite of nut, but the status of *Cladosporium herbarum* is less certain ; the evidence suggests that it may assume a weakly parasitic condition but no more. Possibly this applies also to other associated fungi, for the present investigation does not claim to be exhaustive. Similarly, it cannot be asserted that these fungi exclusively invade oviposition wounds made by the Nut Weevil, for, as Fig. 4 shows, infection sometimes occurs when a nut is very small ; it may later lead to contact infection through the cluster base. The most likely mode of infection at this early stage, if not through a wound, is by the entry of spores through a natural opening in the young nutlet at its tip. Sorauer (1900) mentioned this and considered that the disease most frequently originates in this manner. Possibly this is so when regarded in the sense of a first seasonal appearance in a plantation, but in Britain it does not apply to most individual infections. Small, early-infected nutlets, though not really difficult to find, are not such a prominent feature of the disease as the loss of crop later brought about by infection through the oviposition punctures of the Nut Weevil. A fresh wound from whatever agency would presumably be liable to infection, but this insect is evidently the most common wound agent, as *S. fructigena* is the most common parasite. Only rarely have nuts been observed where primary infection is not attributable to the presence of an obvious wound or a mechanical fault in the shell. An infection of doubtful origin is illustrated in Fig. 5, where the *Fusarium* sp. concerned (distinct from *F. lateritium*) seems to have invaded the nut from a point in the soft, irregular tip where the two halves meet in the suture ; such nuts are, however, usually empty, and the direct parasitism of the associated fungus is problematic. On the other hand, the difference between this type of invasion and that through a mechanical fault at the tip of the shell may, perhaps, be merely a matter of degree.

Good commercial control of the disease could evidently be expected if some means could be found of preventing oviposition by the Nut Weevil. In the spraying experiments described, sprays containing lead arsenate were only partly successful even when thoroughly applied three times in sequence, possibly because the activities of the Weevil are not curtailed until it has consumed enough of the stomach poison to kill it, which may require a long time. A fairly quick and certain " kill " seems desirable.

What was originally approached as a pathological problem, possibly requiring the use of fungicides, has thus resolved itself largely into an entomological one requiring further investigation of the habits of *Balaninus nucum* as a basis for the intelligent choice and application of a suitable insecticide.

It is apparent from field observation that entire clusters of cultivated hazel-nuts may drop prematurely without any evidence of rotting. Inadequate pollination or the effects of prolonged drought are suspected of being concerned, and the loss of crop can be severe.

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SUMMARY.

Rotting and premature dropping of cobnuts and filberts result in severe losses of crop in commercial plantations. The rot was proved by experiment to be chiefly due to wound-parasitism by *Sclerotinia* (*Monilia*) *fructigena* following oviposition punctures by the Nut Weevil (*Balaninus nucum*) in the soft-shelled nuts early in July. Certain other wound-parasites, viz. *Botrytis cinerea* and *Fusarium lateritium*, were also concerned to a minor degree.

The use of lead arsenate sprays resulted in some reduction in rotting through control of the activities of the Weevil, but protective fungicides were not effective against the pathogen.

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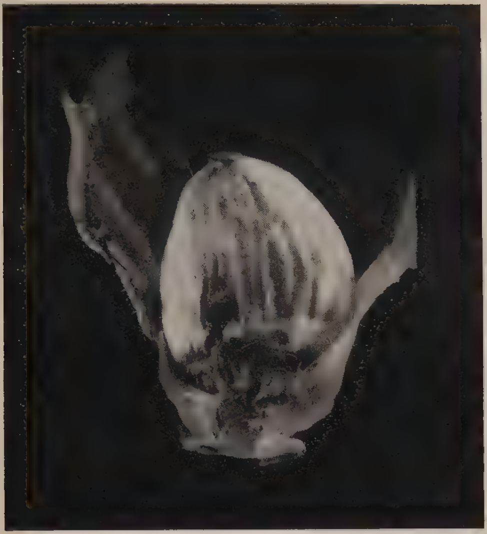

Fig. 1.

Cobnut rotting around oviposition puncture of Nut Weevil.

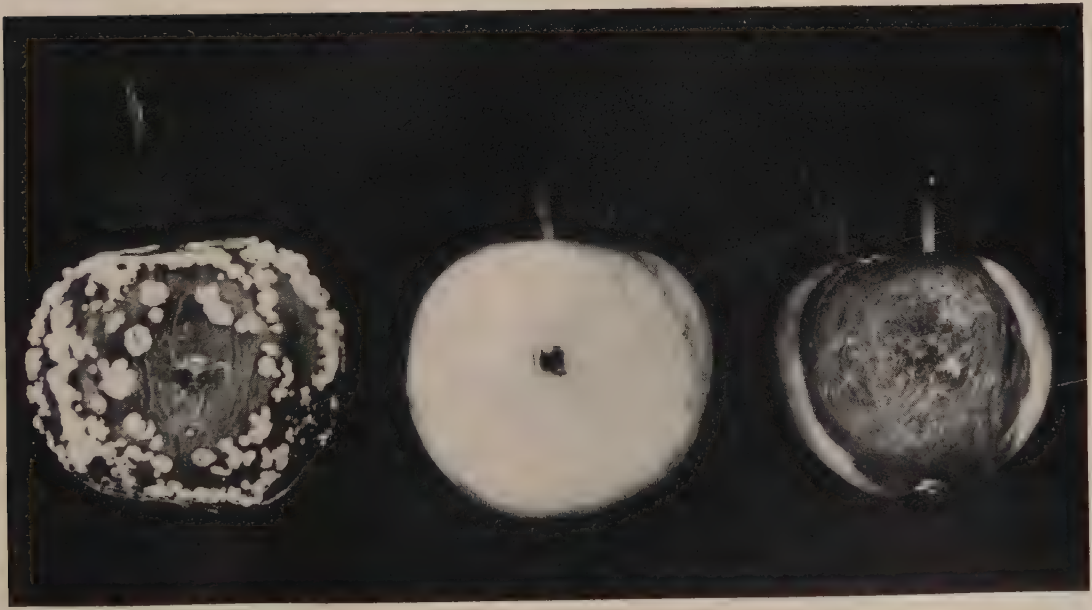

Fig. 2.

Isolation of pathogens of cobnuts in James Grieve apples.
Left: *Monilia fructigena*. Centre: uninoculated control. Right: *Botrytis cinerea*.

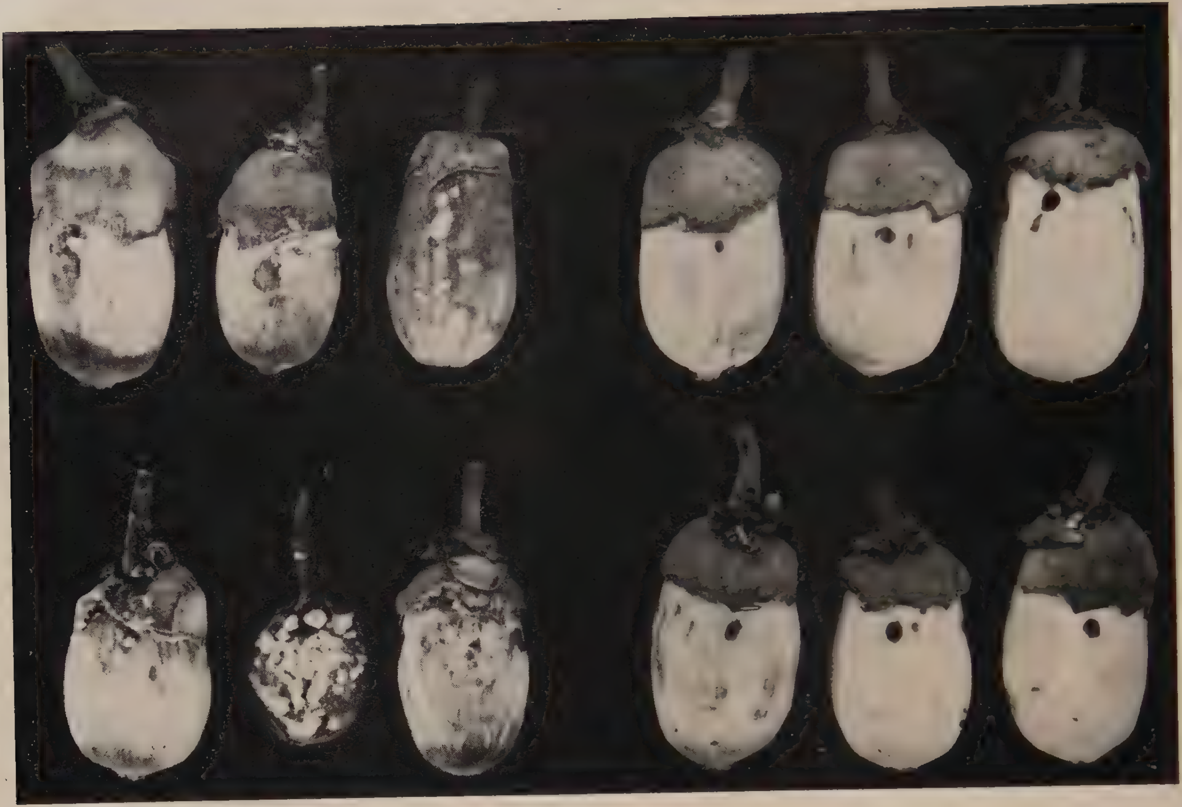

FIG. 3.
Pathogenicity of *Monilia fructigena* on cobnut. Left: inoculated. Right: pierced but not inoculated (distilled water only). Note slight shell discoloration, probably due to sterilization with alcohol.

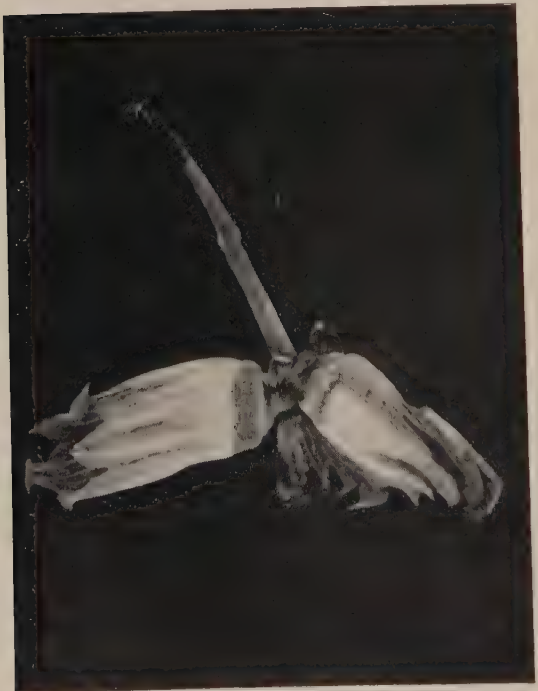

FIG. 4.
Contact infection through the cluster base from a small, early-infected nutlet.

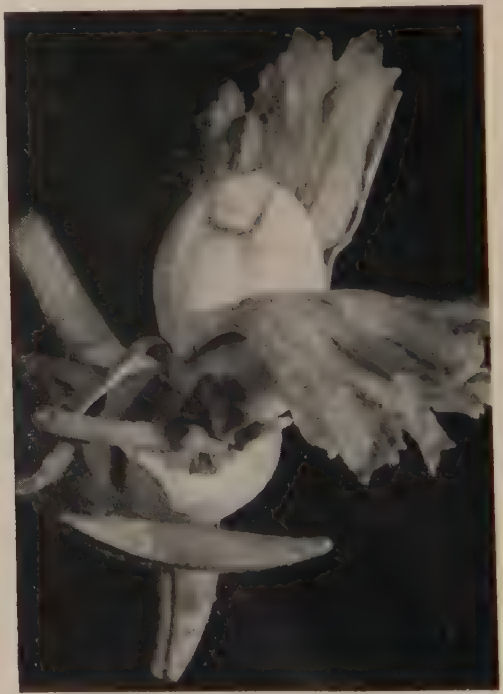

FIG. 5.
Fusarium sp. developing on a cobnut shell apparently from a point at the tip of the suture; there was no obvious wound.

BROWN ROT OF APPLES: FUNGICIDE TRIALS, AND STUDIES OF THE RELATIVE IMPORTANCE OF DIFFERENT WOUND-AGENTS*

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LITERATURE, AND HISTORY OF PRESENT INVESTIGATIONS.

THE comprehensive investigations made by Wormald and others into the Brown Rot diseases of fruit trees were the subject of a monograph (Wormald, 1935), which reviewed existing information on the nomenclature and geographical distribution, host plants, morphology, physiology, mode of parasitism, and control of the Brown Rot fungi, and provided an extensive bibliography of the subject. It was made clear that Brown Rot of apples (*Sclerotinia* (*Monilia*) *fructigena*) in nature arises almost invariably by wound parasitism, and only exceptionally, in the laboratory, has it been noted as a direct parasite through the lenticels (Horne, 1933). The types of wounds mentioned included damage by insects such as Codling Moth, Apple Sawfly, and wasps; bruises produced by hail; cracking after weather changes, Scab-infection, and the application of damaging sprays; and the tearing away of the stalk from the flesh by careless picking. Indirect measures of control were recommended, such as the removal and destruction of sources of infection, and the avoidance of wounds; it had already been demonstrated (Grubb, 1921; Moore, 1932) that good control of Apple Scab by spraying also yielded some control of Brown Rot. Wormald further concluded that " no direct measures of control are practised against these fruit rots ".

Such of this information as was relevant to fruit plantations had been made available to growers in various ways, but numerous complaints were received during the recent war of the alarming increase in losses from Brown Rot in the main fruit-growing areas of the country. The most likely explanation was a lowering of the standards of fruit production as a consequence of labour shortage, but as it was possible that some new factor concerning the disease would be brought to light, a joint survey was made of some of the farms in one of the affected areas. The findings of this survey were published (Wormald and Moore, 1945), and opportunity was taken to re-state relevant information concerning Brown Rot control. No new factors had been discovered, but it was deemed necessary to emphasize certain of the recommended control measures in the light of the survey, for it had shown that branch- and spur-cankers had hitherto been underrated as a source of infection. Protective treatment with the standard fungicides was not advocated; lime-sulphur had been used for years in the normal spray-programme without effecting any noticeable direct control, and Bordeaux mixture had proved too harmful for apple trees (Moore, 1930, 1932, 1938). The writers stated that the potentialities of the new organic fungicides were unknown: " Whether spraying (or dusting) with some of the newer fungicides offers any more hope is at present an open question that can only be decided by field trials," and it was to investigate these potentialities that the experiments first to be described were undertaken.

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FUNGICIDE TRIALS, 1945-46.

EXPERIMENT I, 1945.

Method. The trees available were 13-year-old Cox's Orange Pippin on M.IX planted 12 ft. square, and the four treatments were randomized each on single trees in fourteen replications, each a square of four trees. The treatments, comprising a representative selection from some of the promising new types of fungicides, were as follows :

1. A dispersion in methyl cellulose of commercially pure ferric dimethyldithiocarbamate (F.D.D.C.) at 0·2 per cent.*
2. Tetramethylthiuram disulphide at 0·1 per cent. T.M.T.D.S. in commercial form as a wettable powder.
3. Phenyl mercury chloride (P.M.C.) at 0·005 per cent., in commercial form on china clay, as a wettable powder.
4. No spray.

All the trees, hitherto well sprayed with lime-sulphur for Scab, were sprayed with lead-arsenate suspension for Codling Moth control and were allowed to dry before application of the experimental sprays on 13th June from a 2 h.p. mobile machine working at about 300 lb. pressure per sq. in. The fruitlets were mostly ¾ to 1 in. in diameter.

Records. These were made weekly from 13th August until picking time, 14th September ; all fruits infected with Brown Rot were picked on each occasion and classified into primary and contact infections. There was a light to moderate attack of Codling Moth, and some Scab. The total of rotted fruits was expressed as a percentage of the total number of fruits in the crop, including those picked at harvest.

Results. A summary is shown in Table I ; clearly none of the treatments had reduced infection. It is of interest that contact infections averaged some 6 per cent. in these circumstances.

TABLE I.

Percentage Brown Rot in relation to spray treatment, 1945.

Spray.	Av. no. fruits per tree (14 trees).	% Brown Rot.	
		Primary infections.	Primary + contact infections.
F.D.D.C. ..	185	17·4	23·8
T.M.T.D.S.	163	15·9	21·8
P.M.C. ..	167	13·7	19·6
Nil ..	205	12·3	16·9
		Mean : 14·8	20·5

EXPERIMENT II, 1946.

Method. Similar trees (12 years old) were used on another plot, and a similar arrangement was adopted, but in this case there were seven replications.

The four treatments were the same except for T.M.T.D.S., which was replaced by an 80 per cent. dispersion of zinc dimethyldithiocarbamate (Z.D.D.C.) at 0·2 per cent., more recently available for comparison with the ferric salt, which this time was also an 80 per cent. dispersion used at 0·2 per cent. Both materials were supplied as wettable powders through the courtesy of I.C.I. Ltd. A spray containing zinc-lime had shown some promise against Brown Rot of peach in America (Roberts and Pierce, 1932).

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* The writer is indebted to Mr. R. W. Marsh of Long Ashton Research Station for a supply of the pure material, and to Dr. H. Shaw, who compounded it for use.

All trees were well sprayed up to and including Petal Fall to control Scab and insect pests; then in order to allow subsequent freedom for the development of Codling Moth attack and other blemishes that encourage Brown Rot infection, the trees remained unsprayed till 15th July (a month or so later than in 1945 to diminish the interval between spray deposition and the start of infection), when the fungicides were applied as in 1945. The applications were repeated on 29th July to renew their protective value over the long period during which Brown Rot infection can occur.

Records. The method of recording adopted in 1945 is open to the criticism that all infections were removed from the experimental trees soon after they occurred, thus limiting the likelihood of contact infection, and also removing inoculum. In 1946, therefore, while the weekly recording was maintained as far as possible, it was confined to dropped fruits. One season's experience of this method, however, showed its defects, for critical recording of soiled and rotting fruits was virtually impossible; the wound sites for the primary infections were very difficult to diagnose, and it was often open to question whether the fruit was infected before or after it fell. There had been no indication in 1945 that infection was hindered by the removal of infected fruits from the relatively few experimental trees in the midst of a plantation where Brown Rot was prevalent. Inoculation experiments on cobnuts *in situ* (Moore, 1949) showed the ubiquity of the Brown Rot fungus, and support the claim that the early removal of infected fruits from only a small area is likely to make little material difference to the Brown Rot status of a plantation.

Results. There was a moderate crop, and a summary of the data, again expressed as percentage Brown Rot in the whole crop, including dropped and harvested fruits, is shown in Table II.

TABLE II.

Percentage Brown Rot in relation to spray-treatment, 1946.

Spray.	Av. no. fruits per tree (7 trees).	% Brown Rot.
F.D.D.C. ..	44	26·0
Z.D.D.C. ..	45	33·6
P.M.C.	51	29·0
Nil	35	30·1

Infection was severe, and was doubtless greatly increased by contact infection. Again it is evident that none of the treatments proved effective in spite of later and duplicated spray applications.

DISCUSSION OF PRESENT POSITION ARISING FROM FUNGICIDE TRIALS.

Bennett, Kearns, and Marsh (1944) described a trial on large Cox cordons in Worcestershire in which F.D.D.C. as "Fermate" showed promise in checking Brown Rot, but—to cite two possibly important differences—the fungicide was there used in association with 1 per cent. Grade G oil emulsion, and the attack was a light one, as emphasized by Marsh (1947). He also reviewed recent American results showing control of Brown Rot in cherries and peaches by summer spray treatment with certain new organic fungicides, notably F.D.D.C. as " Fermate ", T.M.T.D.S., and disodium ethylene *bis*dithiocarbamate (" Dithane ").

Recent results obtained at Reading University (Byrde, 1948) show that in a field trial on nine isolated, 40-year-old standard Belle de Louvain plum trees, one application on 23rd June, 1948, of a P.M.C. preparation similar to the one used in the present trials, and at the same strength, reduced Brown Rot infection by about three-quarters compared with that on unsprayed controls, and lime-sulphur at 1 per cent. reduced it by about one-half.

The present evidence suggests that at least some of the new organic fungicides, including phenyl mercury chloride, are unlikely to offer any more prospect than the older, inorganic ones, of controlling Brown Rot by protective spraying. It seems probable that success with summer applications of protective fungicides largely depends on the prevailing circumstances, and, unless persistence and redistribution of the materials are important factors, it may follow a chance timing of the sprays to coincide with a peak period of fresh wound-formation in the fruit.

On the whole, indirect control offers at present a more hopeful approach, and the following experiments were undertaken to study the relative importance of the more common wound-forming agents, and to assess the likelihood of controlling them. Meantime, an allied line of attack is being studied by Marsh (1947) and Byrde (1948) who, following up the successful use of certain arsenical preparations as eradicant sprays in the U.S.A., have demonstrated the possibility of reducing the supply of inoculum by winter treatment of mummied apples and plums with phenyl mercury chloride at a relatively high concentration, 0·3 per cent., or with a mono-calcium arsenite preparation at 0·25 per cent. and 0·4 per cent., all of which depressed the production of spore-cushions in the following summer.

THE RELATIVE IMPORTANCE OF DIFFERENT WOUND-AGENTS, 1946-48.

In the Crimea, Kazanskiĭ (1935) investigated the relationship between infection by *Monilia* and the attacks of certain insect pests on apricots, plums, and apples. The pests included *Rhynchites bacchus* L., the Red Plum Maggot (*Cydia funebrana*), and two species of *Hoplocampa*, *H. flava* on plums and *H. testudinea* on apples. He concluded that *R. bacchus* is the principal agent in the dissemination of *Monilia* in the Crimea, and that the disease can be controlled through control of this insect and in no other way. Fruits containing eggs of the weevil are invariably attacked by *Monilia.* This pest is not of any importance in Britain. The Red Plum Maggot is said to be responsible for 50 per cent. of *Monilia* infection in plums in the Crimea, spores obtaining entry through the insect burrows, but as the larvae cannot develop in infected fruit, they migrate to healthy ones and carry the fungus with them. Both species of *Hoplocampa* are said to render fruits attacked by them practically immune from infection by *Monilia*, this being confirmed by experiment on plums.

In Britain, data are lacking on the relative importance of each wound-agent. Information is necessary for an assessment of crop loss that is :

(i) *avoidable* by careful attention to the control of pests and diseases that predispose affected fruits to Brown Rot infection.
(ii) *unavoidable* in the general hazards of apple growing.

Experiments to obtain data were therefore designed. The avoidable wound-agents studied were: Apple Scab (*Venturia inaequalis*), which may cause fruits to crack during their growth after infection: Apple Sawfly (*Hoplocampa testudinea*), and Codling Moth (*Cydia pomonella*). The unavoidable ones were mostly mechanical i.e. percussion wounds such as abrasions, hail damage, etc., and cracking due to frost and to growth stresses following skin blemishes due to unfavourable conditions, together with occasional bird pecks and the workings of wasps (for this purpose referred to as " similar damage "). Some of these are illustrated in Fig. 1.

Method. The method considered most likely to yield the information desired was to permit free development to each wound-agent separately by omitting from an otherwise complete spray-programme the component necessary for its control. This method is, of course, not applicable to mechanical and similar damage; this is common to all treatments, and is therefore assessable by the use of a complete spray programme. This, then, was the " control ", and four spray treatments were compared, viz.:

1. Complete, including sprays for Scab, Sawfly, and Codling.
2. Complete but for the omission of lead arsenate sprays for Codling.
3. Complete but for the omission of a nicotine + wetting agent spray for Sawfly.
4. Complete but for the omission of lime-sulphur sprays for Scab.

The trees were similar to and growing contiguously with the Cox on M.IX used in Fungicide Trial II in 1946, and were sprayed with the same machine.

The complete spray programme was as follows:

Green Cluster.	Lime-sulphur (2½ per cent.) for Scab, + lead arsenate powder (0·2 per cent.) for Winter Moth caterpillars and Tortricides.
Pink Bud.	Lime-sulphur (2 per cent.) for Scab.
Petal Fall.	Lime-sulphur (1 per cent.) for Scab, + nicotine (0·05 per cent.) and a wetting agent for Sawfly.
Fruitlet.	Lime-sulphur (1 per cent.) for Scab.
Mid-June and end of June.	Lead arsenate powder (0·2 per cent.) for Codling.

These sprays followed the routine use of tar-oil in winter against aphids and Apple Sucker on all trees.

Records. These were made as nearly as possible weekly from the time Brown Rot infection became established (usually in July) until harvest. In 1946 affected fruits were allowed to drop as in Fungicide Trial II, 1946, but in 1947 and 1948 they were removed from the trees as in Fungicide Trial I, 1945, and classified according to the wound origin of the rotting.

Results. The data, summarized in Table III, are based on the total number of fruits per tree from the time (July) Brown Rot infections began, and they include harvested fruits. The values for the mean total number of fruits per tree for any one year do not differ significantly; it must be appreciated that they include all fruits, whether rotting or not, from the time they were about half-grown, so that they would not reflect crop *loss* as do the figures for harvested fruit in relation to total fruit (percentage loss). On the other hand, the data for total percentage of rotted fruit showed significant differences as between the higher two and the lower two values, i.e. most rotting followed the omission of Codling and Scab sprays

TABLE III.

Analysis of rotted and non-rotted fruits in relation to specific wound-agents, 1946-48.

Treatment.	Mean % Rotted Fruit. Wound Source: Mech.	Codling.	Scab.	Others.	Total % Rotted.	Mean % Non-Rotted Fruit. Damaged by: Mech.	Codling.	Scab.	Others.	Total % Non-rotted	Mean total No. fruits per tree.	Mean total crop harvested per tree.	% loss (all sources) July to September.
1946.													
1. Complete spray ..	10·1	0·5	0·8	—	11·4	—	—	—	—	88·6	61	43	29·5
2. Minus Codling sprays	21·4*	7·0	0·5	—	28·9	—	—	—	—	71·1	85	28	67·1
3. Minus Sawfly spray ..	6·4	0·3	0·5	—	7·2	—	—	—	—	92·8	55	42	23·6
4. Minus Scab sprays ..	12·2	1·7	5·7	—	19·6	—	—	—	—	80·4	54	36	33·3
1947.													
1. Complete spray ..	2·1	4·9	0·6	2·0	9·6	10·1	20·1	1·2	—	90·4	189	153	19·0
2. Minus Codling sprays	3·6	15·7	0·7	2·5	22·5	4·7	32·6	0·8	—	77·5	215	107	50·2
3. Minus Sawfly spray ..	2·5	4·5	0·5	1·0	8·5	6·4	24·6	2·2	—	91·5	196	165	15·8
4. Minus Scab sprays ..	6·5	4·8	4·1	1·4	16·8	12·0	10·6	19·3	—	83·2	209	150	28·2
1948.													
1. Complete spray ..	4·7	1·0	0·2	0·3	6·2	25·9	7·4	4·9	1·8	93·8	85	64	24·7
2. Minus Codling sprays	4·8	7·1	0·1	0·3	12·3	16·7	39·5	2·4	0·7	87·5	139	59	57·6
3. Minus Sawfly spray ..	4·2	0·7	1·1	0·3	6·3	28·9	7·5	3·8	4·5	93·5	109	62	43·1†
4. Minus Scab sprays ..	3·3	0·0	18·8	0·0	22·1	3·1	0·1	74·4	0·0	77·8	87	8‡	90·8

* Probably included many fruits attacked by Codling.
† Mostly due to heavy, unexplained loss on one tree.
‡ Most of the crop dropped early through Scab.
N.B. No data are available for the missing values.

respectively, and here, therefore, the loss of crop was obviously greatest. In addition, in 1946 but not in 1947 or 1948, the omission of Codling sprays resulted in significantly more rotting than the omission of Scab sprays. In the specific analysis of rotted fruit, the omission of either Codling or Scab sprays resulted in a significant increase in rotting every year.

DISCUSSION.

The following three major points clearly emerge :

1. *Codling Moth and Apple Scab were the chief precursors of Brown Rot.*

Seasonal conditions were a potent factor in determining the relative importance of each. In 1946 and 1947 Codling was the more important, and a similar general impression is gained from observations over many seasons in commercial plantations. Rotting fruits from this cause are often specially prominent on trees near fruit packing sheds, where Codling tends to predominate. In the dry 1947 season, Codling infestation was very severe and included a second generation, though this was not responsible for more than some 2-3 per cent. increase in Brown Rot on the tree (from original field data, where the two generations were separately classified). It is very likely, however, to increase rotting in store. This second generation was the chief cause of the higher values for Codling among the non-rotted fruit, and it was apparently little affected by spray-treatment applied to control the first generation. Codling was severe also in 1948, but only a relatively small percentage of infested fruits rotted, as is seen by the high value for non-rotted infested fruits. Infestation occurred over an unusually long period, possibly because of the wet summer. Evidently such variations in seasonal conditions are a factor in the extent to which rotting occurs even where wound sources are abundant. If a wound is not infected while still fresh it may produce callus and is then likely to remain uninfected, though a vulnerable crack may develop following subsequent growth.

Scab-infection was exceptionally severe in the wet 1948 season, and provided a striking example of crop loss on its own account. Most of the fruits on trees not sprayed with lime-sulphur (treatment 4) dropped through Scab in June, thus prematurely removing them from the arena for Brown Rot infection. The figure (18·8 per cent.) for rotting through Scab is thus lower than might be expected in relation to that (74·4 per cent.) for their non-rotted fellows.

2. *Apple Sawfly was not a factor of importance in the incidence of Brown Rot.*

The disease does not usually become troublesome until the latter half of July, by which time fruitlets attacked by Sawfly have either dropped or are dried up and no longer susceptible to rotting.

The immunization theory put forward by Kazanskiĭ (1935) is therefore not likely to apply to apples under British conditions. Persistent fruitlets attacked by Sawfly followed by the Brown Rot fungus, producing spore-cushions, have sometimes been encountered and these could act as sources of infection. Sawfly attack could conceivably also lower the incidence of Brown Rot contact infections by thinning a heavy fruit set.

3. *Mechanical and similar damage was on the whole only a minor factor in the incidence of Brown Rot on the tree.*

It amounted to no more than 4 per cent. or so in 1947 and 1948 when rotting fruits were picked from the trees and there was no confusion as to the source of the wound such as occurred in 1946 on dropped ones. This value might on occasions be greatly increased; a severe hailstorm in July has been known to damage growing apple fruits severely enough to promote wholesale rotting, and other instances depending on particular circumstances could be cited, e.g. cracking following Sun Scald injury (Moore and Rogers, 1943), and the severe attacks by birds and wasps in 1949. High values among the non-rotted fruits in 1948 were mainly due to "weather cracking" of the kind illustrated in Fig. 1. The cracks mostly callused before Brown Rot infection developed.

General Comments.

An unexpectedly low or high value in certain instances in Table III is explained by reference to another value in the same series. For example, in 1946 a relatively high value of 21·4 per cent. rotted fruits was recorded following mechanical damage. Clearly, many Codling-infested fruits were included, but it was not possible to classify them accurately as such on dropped specimens in an advanced state of rotting, so they were included in the category "mechanical and similar damage". Other apparently aberrant values probably owe their origin to fruits each bearing symptoms of more than one kind of damage; these were classified according to the predominating wound agency.

DISCUSSION OF THE EVIDENCE IN ITS RELATION TO CROP LOSS UNDER COMMERCIAL PLANTATION CONDITIONS.

These experiments were promoted less to provide data on what occurs in the average fruit plantation than to establish a standard of relative losses of crop under controlled conditions. The actual losses in the commercial plantation could be determined only by an investigation carried on for several seasons *in situ*, and naturally the results would vary with the quality of farm management and with the district and seasonal conditions. Meantime, the present data serve incidentally as a rough guide to what might be expected.

Taking the mean of the three seasons, the relationship between spray treatment and percentage of rotting was as follows:

Complete Spray.	Minus Codling Sprays.	Minus Sawfly Spray.	Minus Scab Sprays.
9·1%	21·2%	7·3%	19·5%

It must not be overlooked that each figure includes a value for rotting through wounds that proved uncontrollable by spraying; this, represented by the 9·1 per cent. value for the complete spray, must therefore be deducted from each of the others to determine the losses controllable by specific spray treatment. The data include only a one-third allowance for unlimited contact infections (in 1946); these would increase the rotting by a variable amount each year according to circumstances. Where the crop is heavy, the number of contact infections would naturally be enhanced because the fruits would be closer together on the trees. An illustration of this is seen in Fig. 2. In 1945, with a heavy crop, the figure was some 6 per cent., but in this case it was artificially limited by the periodic removal of primary infections by hand, and Scab, and to a less degree, Codling had been confined to fairly low levels by appropriate spraying. Given free play for contact infections and a moderate attack by both pests, this average figure for "contacts" might well be doubled.

From the data available, it can be deduced that the average *unavoidable* crop loss through rotting on the tree in normally cropping Cox's under good commercial conditions would probably be about 10 per cent. This value includes some allowance for contact infections, as well as for rotting through wounds made by mechanical agency and by the pests that survive even good spray treatment.

The additional figure for *avoidable* loss will obviously depend much on the quality of the farm management. Where no sprays for Codling and Scab are applied, the average avoidable loss on the tree through mostly primary Brown Rot infection can lie between 20 per cent. and 30 per cent. of the crop, according to the present experiments ; in the three seasons under review this loss was due in roughly equal proportions to the agency of Codling and Scab, though in general Codling is probably the worse offender.

Attacks of aphids and caterpillars of various kinds, which were controlled on all trees in these experiments by the routine use of tar-oil in winter and the inclusion of lead arsenate in the Green Cluster spray, may also on occasion add their toll.

Contact infections progressively enhance the loss from primary infection and, where the fruit is carelessly handled at and after picking and blemished apples are stored, further losses through rotting must be expected in addition to those sustained before picking. Hence considerably more than half of the total crop could be wasted through rotting alone under such conditions. Moreover, it has been shown that uncontrolled Scab can itself cause severe early fruit loss when the weather is favourable for infection, Brown Rot then destroying much of what remains. Codling likewise is itself responsible for crop loss.

Fortunately, such aggregate losses represent what might be encountered in hypothetical circumstances, for in good commercial practice Codling and Scab are not allowed unrestricted scope. On the other hand contact infections can develop freely on the farm because it is not feasible to remove primarily-infected fruits from the trees. These two opposing factors must be taken into account in arguing from the results of these experiments to commercial practice, where also the variety of apple concerned would bear on the issue.

The salutary fact remains that the *avoidable* loss in Cox's through primary rotting alone can be some two or three times as much again as the unavoidable loss, under conditions where each is given free scope. Hence, though direct spray treatment may fail, indirect spray treatment, coupled with as much observance as is practicable of the general principles of plant hygiene, is evidently sound economic practice for Brown Rot control.

It should be pointed out that plums and cherries present rather a different problem from that of apples, in that *M. fructigena* attacks these fruits chiefly when they are ripe or nearly so. At that stage they more nearly parallel the so-called soft fruits in their vulnerability to mechanical damage, as well as to the ravages of birds, wasps, etc., and it is therefore highly probable that the part played by these largely unavoidable injuries in predisposing the fruits to rotting is relatively much more important in plums and cherries than in apples.

ACKNOWLEDGMENTS.

The writer is indebted to Miss Margery Bennett for much valued assistance in supervising these experiments and the recording and computing work involved ; to Mr. R. D. Souter for sustained and accurate help in the onerous task of recording ; and to Miss J. M. S. Thom for statistical analysis of the data.

SUMMARY.

Certain of the new organic fungicides showed no promise of controlling Brown Rot of apple, *Sclerotinia* (*Monilia*) *fructigena*, when used as protective sprays.

Experiments were made to determine the relative importance of certain wound-agents functioning as precursors of the disease, and to assess the proportion of total rotting due to controllable causes. Codling Moth and Apple Scab proved to be the chief agents concerned ; Apple Sawfly was not of material importance; mechanical and similar damage played a relatively minor part. It was concluded that avoidable losses from mostly primary rotting, which can largely be prevented by spray treatment designed to eliminate the wound-agents, can amount to two to three times as much again as unavoidable losses, though for reasons given this extreme difference is not likely to arise in good commercial practice.

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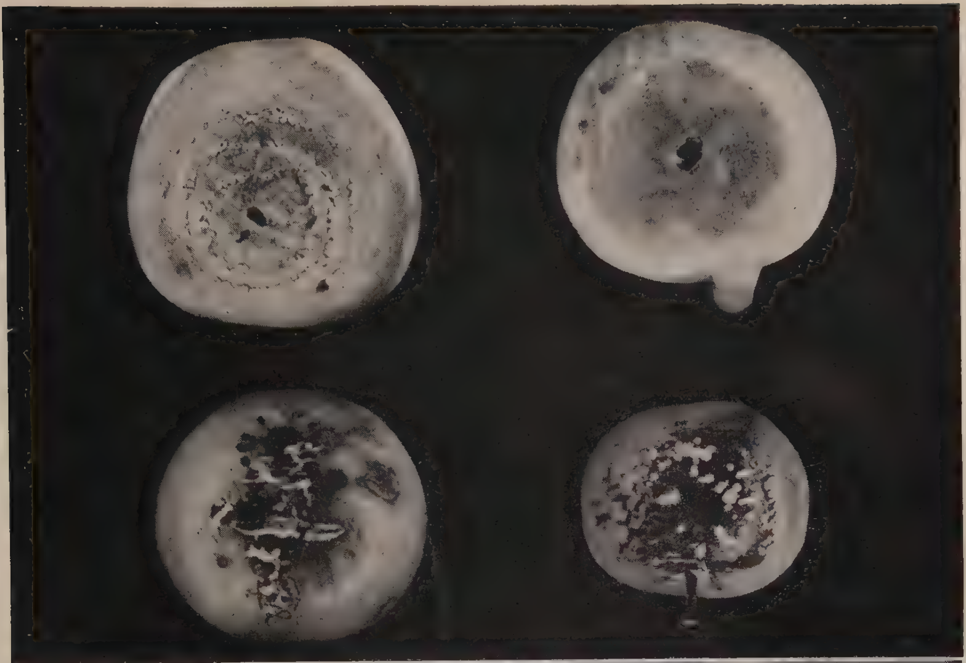
(*Received* 13/5/49.)

Brown Rot of Apples: Fungicide Trials and Studies of the Relative Importance of Different Wound-Agents. By M. H. Moore.

PLATE I.



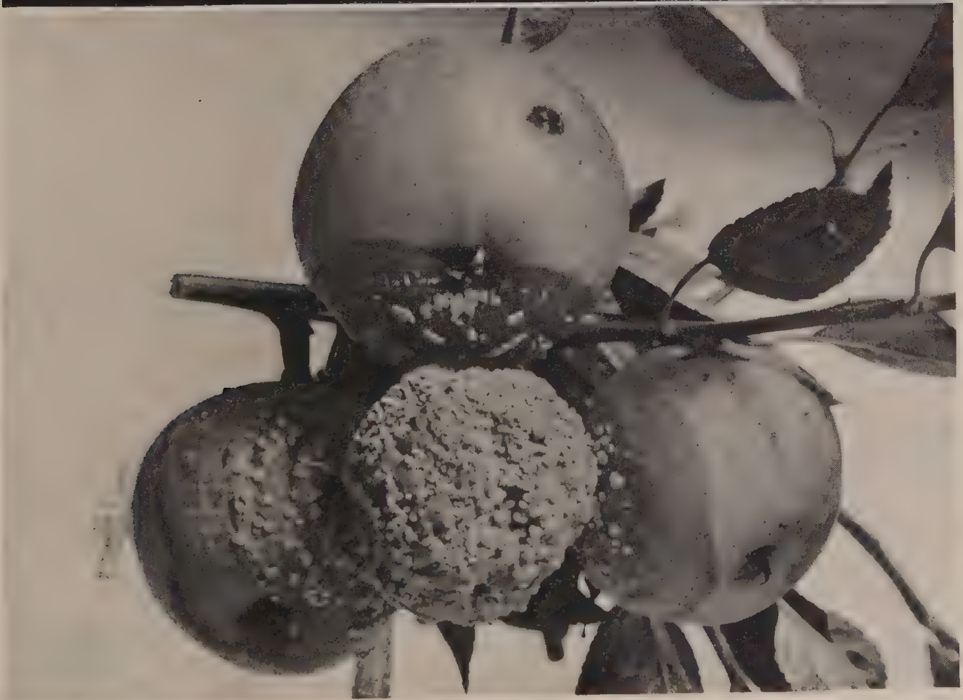


FIG. 1.

Some of the types of wound (top, Allington Pippin; bottom, Cox's Orange Pippin) from which infection by *Monilia fructigena* and other wound parasites can develop. Top left: hole made by larva of Codling Moth. Top right: bird peck. Bottom left: cracks following early Scab-infection. Bottom right: growth cracks and russeting following frost or other unfavourable weather conditions.

FIG. 2.

Contact infections of *Monilia fructigena* developing from primarily-infected central fruit in crowded crop of Allington Pippin.

CITRUS GROWTH STUDIES

II. FRUIT-GROWTH AND FRUIT INTERNAL QUALITY CHANGES*

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THIS is a further paper reporting the results of extensive investigations on various phases of growth of the citrus tree, carried out from 1937 to 1940, under mild subtropical climatic conditions in a summer rainfall area. The author (1949b) has pointed out that our understanding of the growth physiology of the citrus tree hitherto has been based mainly on research on trees growing in climates more severe than those experienced in the major citrus-growing areas in the Union of South Africa, and under winter rainfall. A large proportion of the world's citrus is grown in climates ranging from mild subtropical to tropical, and these papers are offered as contributions to our knowledge of the behaviour of the citrus tree under those conditions.

FRUIT-GROWTH.

Brief anatomical studies of the citrus fruit have been described by European botanists in the past century, but it is only in very recent years that the growth of the fruit itself in relation to environmental conditions has received attention from citriculturists.

Size of fruit is a very important factor in commercial citrus-growing ; the larger the fruit the greater the yield per tree as measured by packed cases, provided the set or number of fruit carried by the tree is normal. Some markets pay a premium for large fruit, while others prefer medium sizes ; small sizes are usually sold at a discount. The primary factor determining fruit size is variety, but within any given variety a number of factors affect the size of the fruit at maturity. Usually young trees produce larger fruit than trees which have come into full bearing, and older trees with a small crop produce larger fruit than trees of the same size having a normal crop, while trees carrying a very heavy crop produce smaller fruit.

Hodgson (1947) lists reasons for small fruit as follows : Florida—heavy annual crops, inherently small-fruited varieties, use of rootstock tending to reduce sizes. California—deficiency in total heat units available, a series of heavy crops, widespread lack of vigour. Batchelor (1948), in discussing the problem of small-size oranges in California, shows a high probability that years of heavy production are also years of small sizes, but points out that it is uneconomical to thin the heavy crops to induce larger sizes, because the reduction in total crop and the cost of thinning are not compensated by the small increase in price obtained for the larger sizes. Anything which results in subnormal growth and condition of the tree adversely affects size of fruit : thus various factors which impair the metabolism of the tree such as zinc deficiency, dust and oil spray coverings, and fertilizer starvation, all result in smaller fruit. In South Africa, it has been shown that virus-infected " greened " and " stem-pitted " trees produce markedly smaller fruit than healthy trees.

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* Paper read at the Forty-seventh Annual Meeting of the South African Association for the Advancement of Science, Kimberley, 4th July, 1949.

It is, however, with fruit-growth and its relation to fruit size under what would in practice be considered normal conditions of tree growth that this study is concerned. The work of Waynick (1928), (1929), (1932) on Valencia oranges in California shows that the most important factor determining growth and final size of fruit is the water available in the root zone during the period of most rapid fruit-growth, which is during the second half of the first seven months after blossoming. Fruit which has not reached average size by this time will not reach average size by picking time seven months later. Number of fruits borne is not alone responsible for small sizes within ordinary limits of the productive capacity of the tree. Permanent effects result from retardation of growth during the critical period, whether due to shortage of soil moisture, desiccating winds, or heat. Waynick further found that with the exception of fruits initially very large, those which are smaller four months after blossoming grow more rapidly than those then larger, and they equal the latter in size at picking time. He states that fruit from trees on the sandier soil types definitely reaches full size earlier than does fruit from trees on heavier types while, irrespective of soil type, fruit inside the tree continues to increase in size for some time after outside fruit has stopped growing.

Furr and Taylor (1939) found that changes in the apparent growth rate of lemon fruits serve as an excellent index of the relative water deficit of the tree. If trees suffer a deficit to such an extent that the leaves roll, there is a pronounced reduction in final size of fruit on all soils; but if the deficit is only moderate to slight before water is applied, then the final size of fruit is smaller only on light and sandy soils. Harding *et al.* (1940), (1945), in their very comprehensive work on seasonal changes in Florida oranges and grapefruit found that the greatest increase in diameter of fruit precedes the attainment of prime eating condition (which for Valencias in Florida is only nine months after blossoming) and thereafter growth is much less rapid. The size of fruit is influenced by soil type, age of tree, cultural practices, and rootstocks, fruit from trees on rough lemon being larger than fruit from trees on sour orange stock.

The work of Stewart and Hield (1949) shows a definite increase in fruit size with oranges and grapefruit, without an increase in total crop, due to spray applications on normal trees of the hormone, 2,4-D. Growth responses are most marked just after blossoming time, the effect being a slight reduction in the number of fruit resulting, with a final increase of one pack size accompanied by delayed maturity of the fruit. Spraying with hormone is not yet recommended as a commercial cultural practice, because it is not known what cumulative effect there may be on the tree, neither is it established that the cost is justified by increased returns.*

EXPERIMENTAL.

The fruit-growth data herein discussed were obtained on Valencia orange trees, on rough lemon and sweet orange stocks, 5 years old in 1939, and just coming into commercial bearing. Two series were obtained, the one referred to as " at the pits " being from the two trees in the Citrus Variety Orchard on which the intensive general growth studies were conducted, and the other from the adjacent Citrus Fertilizer Orchard.

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* Since this paper was submitted for publication, several articles of great practical importance have appeared in Vols. 34 and 35 of *The California Citrograph*. These further discuss the importance of efficiency of irrigation, of irrigation methods, and of soil water relations as influencing the size of oranges. Among the authors are M. R. Huberty, C. P. Teague, and B. E. Yorick.

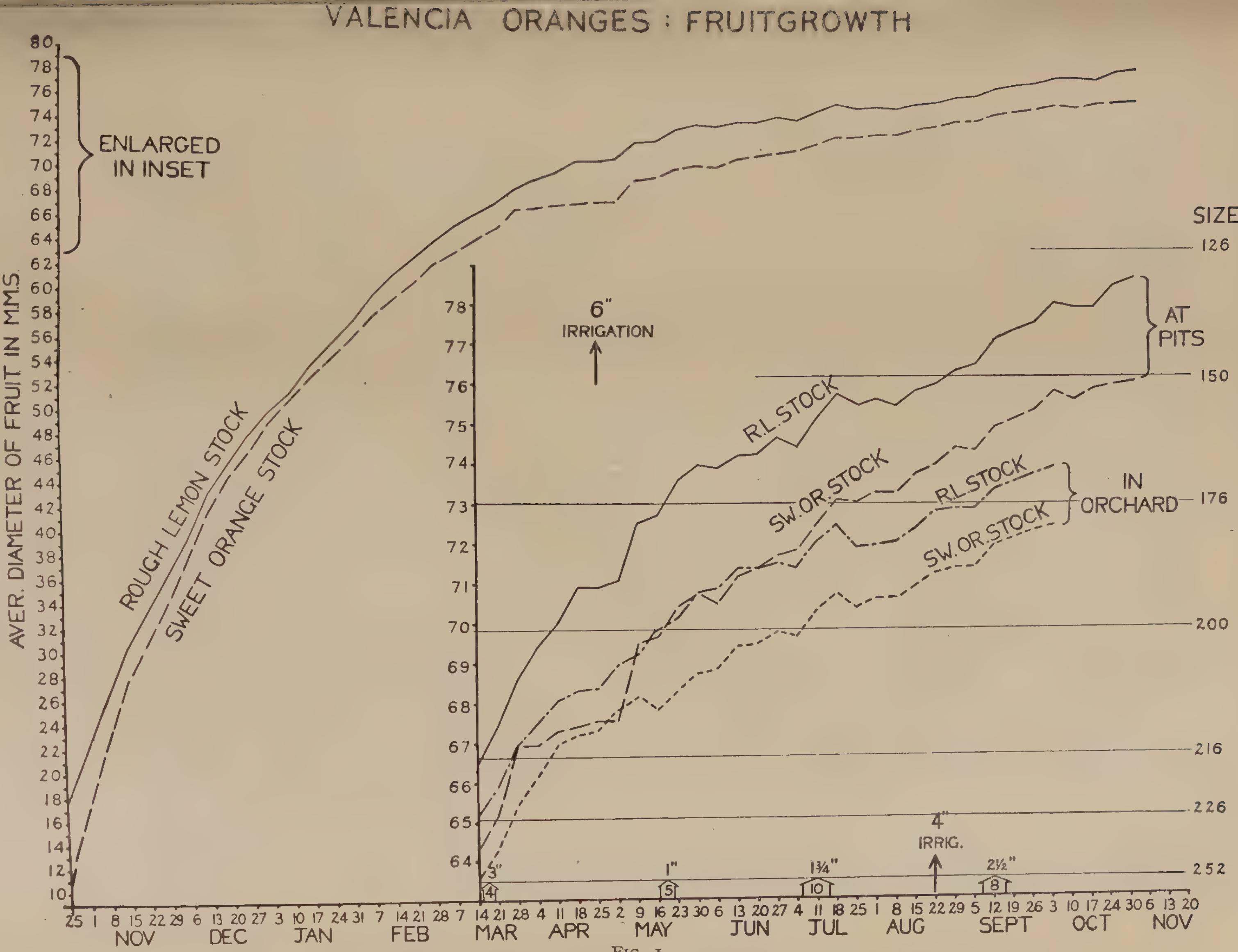

Fig. 1.

Growth curves of Valencia oranges from trees on sweet orange and rough lemon stocks in two orchards.

At Nelspruit, Valencia trees are usually at the height of their blossoming period at about the third week in August and may often remain in full blossom as late as the 12th September, as they did in 1939. At that time the previous season's crop is still on the tree, being harvested from August to October, i.e. from 12 to 14 months after fruit-set. On each of the trees at the pits, one on rough lemon and the other on sweet orange stock, twenty external fruits around the tree were tagged shortly after they had passed pea-size, six weeks after being set. In the Fertilizer Orchard, four adjacent trees on each of the two stocks had five fruits tagged on each tree, making twenty fruits per stock, and they were tagged six months after setting. The trees in the two orchards are sister trees, growing on the same soil type, but irrigation and cultural practices in the two orchards were not necessarily the same. Weekly measurements of size were made on all the tagged fruits, direct diameter readings being made with a Pillsbury and Compton (1937) fruit circumeter, which is a specially graduated steel tape running through a slide giving vernier readings. The averages of the readings from each set of twenty fruits are plotted in Fig. 1, which shows the growth for a twelve month period for fruits on trees at the pits, and growth of fruits from the adjacent orchard for the latter eight months of the same period. To accentuate the illustration of the variations in diameter which occurred in the latter eight months, the unit spacing on the ordinate of the graph was trebled in the inset.

The period of summer rains in the 1939-40 season extended from the end of October to the middle of March, with regular effective rains starting again in September, 1940. The rains in the interim period are shown in the graph as total rainfall in inches over a period of days indicated by number. Except for the falls on 14th to 16th March, 7th to 10th July, and 13th to 17th July, all these rains were less than 0·5 in., and can be ignored as being ineffective in replenishing soil moisture in the citrus root zone. Two heavy irrigations, 6 in. on 3rd May, and 4 in. on 22nd August were given to the trees at the pits, while those in the Fertilizer Orchard received light irrigations of 2 in. on 16th May and 22nd August. The trees at the pits did not suffer any water deficit at all from the blossoming period in September, 1939, to April, 1940, while those in the other orchard were subject to a medium deficit in October, 1939, with a further deficit in May, 1940.

The figures on the right hand side of the graph are the numbers of oranges of various sizes which are packed into the standard South African export case, which is the same as used in California. A fruit of size 252 is 2½ in. in diameter, and one of 150 is 3 in. in diameter.

The continuous growth fluctuations of an individual fruit were recorded by means of a dendrograph, shown in Fig. 2, for the nine-month period from January to October. The apparatus used was an older model of that described by MacDougal (1938) and used by him on tree-growth studies, adapted to give accurate readings of an individual fruit during its growth on the tree. Fourteen representative weekly recordings were transferred by tracing to provide a comparative picture of the diurnal fluctuations in fruit diameter during the full period of recording, as well as the relative weekly total increase or decrease. In Fig. 3 the equal spacing between lines at the beginning of each week has no relation to the date of week ending, shown opposite each line. The actual fruit being recorded could not be measured weekly for diameter because of practical difficulties in the adjustment of the dendrograph.

PLATE I.

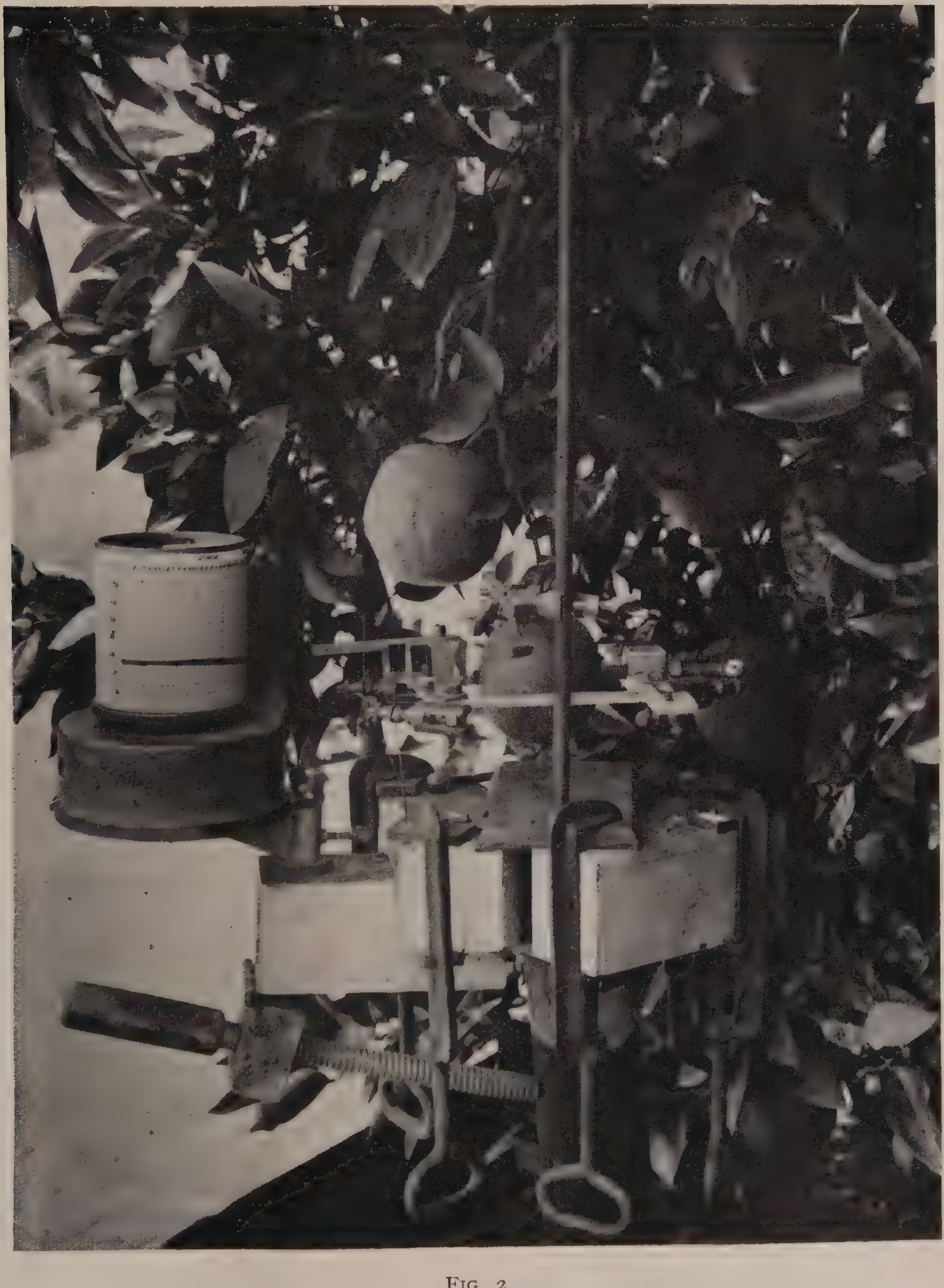

FIG. 2.

Dendrograph installed at the tree to record the fluctuations in diameter of a Valencia orange during its growth.

DISCUSSION.

With citrus the factors which have a major effect on rate as well as on total growth of the fruit, and therefore on final size at maturity, may be divided into two categories. The fixed or inherent factors are variety of fruit, age of tree, rootstock on which the tree is budded, soil type, and climatic conditions. The variable or seasonal factors, usually under the control of the grower, are general health of the tree, the size of crop being borne, the amount of nutrients available in the soil, and, above all, particularly with a normal tree which has set an average crop, water relations during the critical growth period of the fruit from blossoming time in August-September to next March-April.

When it had been determined that for any given tree a direct correlation exists between weight per fruit and fruit diameter within the range of commercial pack-sizes, the influence of yield per tree on size of fruit at picking time was followed over a period of ten years. From the 512 data trees in the Citrus Fertilizer Orchard individual tree records of weights of crop and number of fruits were obtained, the weight per fruit being calculated. On plotting the averages per tree for yield and weight per fruit it was found that over the ten years, high yields showed marked correlation with small fruit in three years, low yields showed a similar correlation with large fruit in three years, and a distinct inverse correlation was found in three years. Thus it might be concluded that while in general small fruit size is associated with heavy crops borne, and vice versa, some unknown seasonal factor upset this correlation in certain years.

Reference to Fig. 1 shows that fruit from trees on rough lemon stock is larger than that from trees on sweet orange, stock being one of the fixed factors. The author (1939) has shown this to be true for young trees at picking time, but it is now shown that the difference in fruit size exists already soon after fruit-set, and continues to the same degree throughout the growth period, the rate of increase being the same for fruit of trees on both stocks. Since the crops borne by young trees on rough lemon stock were some 25 per cent. greater than those of trees of the same size on sweet orange, the fixed effect of rough lemon producing larger fruit could have been to some extent counter-balanced by the variable effect of trees with a larger crop producing smaller fruit. The final difference was about half a pack-size at picking time.

Waynick (1928) records an instance where the fruit from one grove was 2½ pack-sizes, equal to about 30 per cent. in volume of fruit, below the average because the soil moisture was nearer the wilting point than the optimum for 60 days during the critical growth period. It has been mentioned that the trees at the pits were kept well supplied with water during their first seven months of growth, while those in the Fertilizer Orchard suffered one period of deficit and received only limited irrigation to supplement rainfall. The effect of this difference in treatment is shown in Fig. 1, the fruit from the first mentioned trees having been over one pack-size larger at maturity.

That the ultimate fruit size was not affected by a water deficit once the critical growth period was passed is clearly shown by the Fig. 1 graphs for fruit at the pits. The last effective rain fell on 18th March, and as the soil moisture became depleted the fruit ceased increasing in diameter, the trees finally showing morning wilt. A thorough irrigation was applied on 3rd May, and the fruit recovered in size to resume its place in the normal growth curve. Later slight water deficits were reflected in

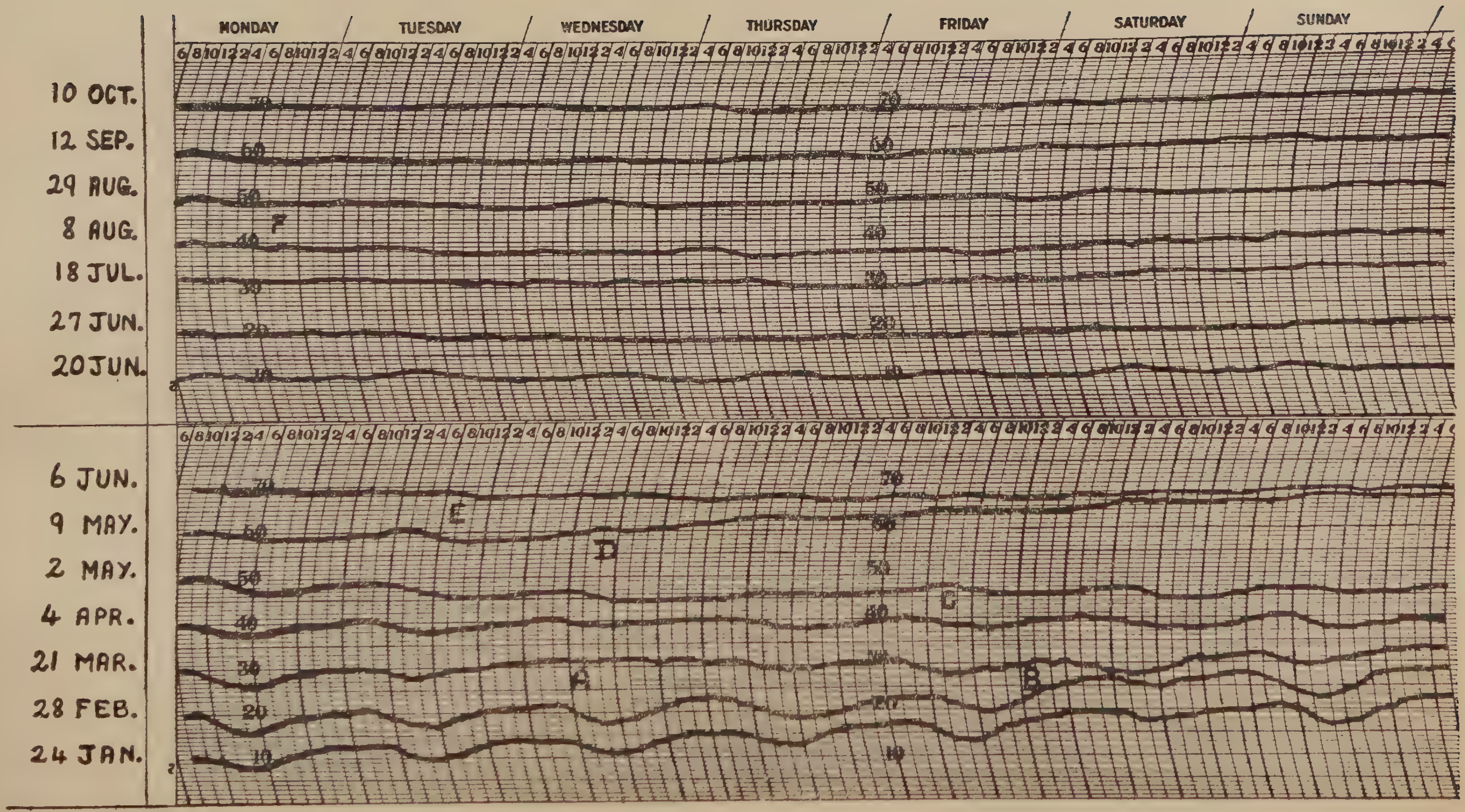

Fig. 3.

Weekly recordings of fluctuations in diameter of a Valencia orange during a nine-months period of its growth on the tree. Dates refer to the day the week ended.

lack of increase or even a slight decrease being recorded when the fruit was measured, but in each instance when the soil moisture was replenished, either by rain or irrigation, the response thereto was that the fruit increased to the size it would have been had the deficit not occurred. After May the fluctuations in size seldom amounted to half a pack-size in degree.

In interpreting the dendrographic recordings shown in Fig. 3 it was found that only relative growth of the fruit can be studied by this means, the absolute growth having to be determined by actual measurement of a number of fruits representative of the tree. Marked diurnal changes in fruit diameter, as shown in the lower two lines, were recorded during the early life of the fruit, this being the period of most rapid growth. The fruit decreased in size during the day till mid-afternoon and reached maximum size in the early hours of the morning. These diurnal changes lessened considerably once the fruit had reached almost full size by May, some eight months after fruit-set. External climatic factors very markedly influenced the diurnal fluctuations ; at point A on the graph no decrease in size during the daytime is recorded, this coinciding with a misty rain which fell throughout that day, while the rapid small changes in diameter recorded at point B are a reflection of unusual dry gusty winds which blew throughout the night of 20th March, causing temporary desiccations of the leaves and fruit while the root zone was still well supplied with moisture.

The dendrographic line for the week ending 2nd May covers a period of severe water-stress for the tree. This is reflected in an irregularity in the diurnal changes during the latter part of the week (point C) and in a lack of total increase in diameter of the fruit. A heavy irrigation was applied on 3rd May, and the effect is shown by the steady rise of the growth line from point D on. As from the middle of June, nine months after fruit-set and when the change from green to yellow colour of the fruit was almost complete, only the slightest diurnal variations in fruit diameter occurred, while by the end of August (point F) time of fruit maturity, even these had ceased to be constant.

Fruit size at picking time is of great practical importance to the citrus grower. With Valencias, the desired sizes bringing the best financial return are those giving from 200 to 252 fruits per packed box for the export market to the United Kingdom, and 100 to 150 for the local market in South Africa. Growers can by seasonal cultural practices such as hormone-spraying, fertilization, and especially irrigation, influence the final average fruit size of their crop, and although this is a new aspect of commercial citriculture which has not received much attention, the practicability of fruit-growth control as regular subsidiary practice is indicated.

FRUIT INTERNAL QUALITY CHANGES.

Sinclair and Bartholomew (1947) point out that the sweet and sour taste sensations given by orange juice are due to the soluble sugars and free acids in the juice. The sensation of sweetness is primarily caused by the proportion of total sugars to total soluble solids (T.S.S.), not the amount of total soluble solids—although in mature fruit this is for practical purposes the same.

As far as can be determined, the first to investigate the changes which occur in the juice of citrus fruits during their growth was Collinson (1913) who found that in Florida grapefruit and oranges, over the four-month period in which the taste of the juice changed from very tart to very sweet, there was a gradual decrease of acid (citric) and a gradual increase in total sugars (sucrose, dextrose, and fructose) as the fruit matured. By taste tests he found that in Valencia fruit a ratio of sugar to acid of 4 : 1 separated very sour from sour, and a ratio of 7 : 1, graded as tart, separated sour from sweet fruit.

Among subsequent studies on the internal composition of citrus fruit, those of Wood and Reed (1938) on grapefruit in Texas, Harding, Winston, and Fisher (1940) on seasonal changes in Florida oranges, Harding and Fisher (1945) on seasonal changes in Florida grapefruit, and Sinclair and Bartholomew (1947) on oranges in California, particularly help towards a clear understanding of the physiological and chemical processes which occur in the citrus fruit during ripening on the tree under the particular climatic environments pertaining. In view of the great influence, emphasized by Webber (1938), of environment, particularly atmospheric humidity, on the rate of growth and on both the external and internal quality of citrus fruit, it was considered desirable to determine the changes which occur in fruit growing under mild winter and summer-rainfall conditions such as prevail in some of the main citrus-growing areas of Southern Africa. Also, no other workers have fully compared the changes in fruit from trees on rough lemon and sweet orange stocks, the former being the only stock used hitherto in the Union and the latter the stock now recommended for new commercial plantings in many of the citrus-producing areas in the Union. The longest period over which other workers had followed the changes in the fruit was eight months, readings being mostly at fortnightly intervals and starting when the fruit was well sized. In this work the determinations were twice as frequent, started earlier on smaller immature fruit, and continued well past the normal picking period to over-maturity.

EXPERIMENTAL.

The fruit used for the fruit quality tests was obtained from young sister Valencia orange trees propagated from a single parent. There were two four-tree plots on each stock ; and weekly samples of eight fruits were picked at random from around each tree, giving composite samples of 32 fruits from each plot. The first sample was taken in February when the 5-months-old fruit was still small and green, and weekly sampling continued till the following January, 16 months after fruit-set, by which time the remaining fruit on the trees was over-ripe and dropping easily.

The following were determined on each fruit sample : weight per fruit, fruit diameter and rind thickness, juice percentage by extraction with a "Sunkist" electric reamer, acid content of juice by titration with sodium hydroxide, and total soluble solids percentage in juice by use of a Brix hydrometer. The weekly values used were averages of the values obtained for the two composite samples from trees on each stock.

RESULTS AND DISCUSSION.

The rate of increase in diameter of fruit.

This was parallel to that in weight per fruit until the colour change from green to yellow started in May, after which the diameter increase rate slowed considerably while weight increase continued at its same rate for a further six weeks, but then slowed to a rate again parallel to that of diameter increase. Thus just before, during and just after the period of maturity, August to October, a direct correlation existed between fruit-size and fruit-weight for average fruit.

Rind thickness.

Rind thickness showed large variations from week to week but increased gradually from the small and immature to the larger and over-mature stage. However, when twice the rind thickness was expressed as a percentage of the total diameter of the fruit, it was found that (except for higher values while the fruit was small and immature) rind thickness remained directly proportional to fruit diameter. Thus, the ultimate rind thickness at maturity was determined in the early stages after fruit-set and was correlated with subsequent fruit-growth. The author (1949a) showed that, at maturity, fruit from trees on sweet orange stock has a significantly thinner rind than has fruit from trees on rough lemon, and here it was seen that this difference existed already at the small immature stage of the fruit and persisted till over-maturity was reached.

The rate of increase in juice percentage.

The rate of increase was rapid from February to June, after which this percentage remained at the same level till a slight drop occurred as the fruit became over-mature in December-January, reflected in a " dryness " of the juice vesicles of the fruit. The attainment of maximum juice content of the fruit was associated with the slowing-up of the growth rate and the colour changes of the rind. Though no difference was found while the fruits were still immature, fruits from trees on rough lemon stock had a lower juice percentage than those from trees on sweet orange during the period of maturity, a difference shown by the author (1949a) to be highly significant. As the fruit reached the over-ripe stage this difference gradually disappeared.

The sugar content of the juice.

This may be considered the most important factor in determining fruit internal quality, being responsible for the " body " of the juice. The changes which occurred in the sugars during growth as measured by the percentage total soluble solids, are shown as graphs in Fig. 4.

The T.S.S. in the juice of fruit from trees on both stocks increased steadily from February to a maximum in August-September (the time when the fruit was regarded as being mature) and decreased at a slower rate till over-maturity in December-January. Attention has not been focused previously on this decrease, but it is of the greatest practical importance, especially in judging when the fruit is in prime eating condition and should be picked for consumption. It is particularly notable that the fruit from trees on sweet orange stock, apart from having a considerably higher T.S.S. content at the immature stage and showing an increase in this difference from September on, maintained its maximum of 10·1 per cent. as against 9·8 per cent. for trees on rough lemon stock for the two-months period August-October before decreasing. It follows that fruit from trees on sweet stock would have been harvested in optimum condition over a longer period than could fruit from trees on rough lemon, and also, even after the decrease in T.S.S. had started, maintained a better eating quality till January.

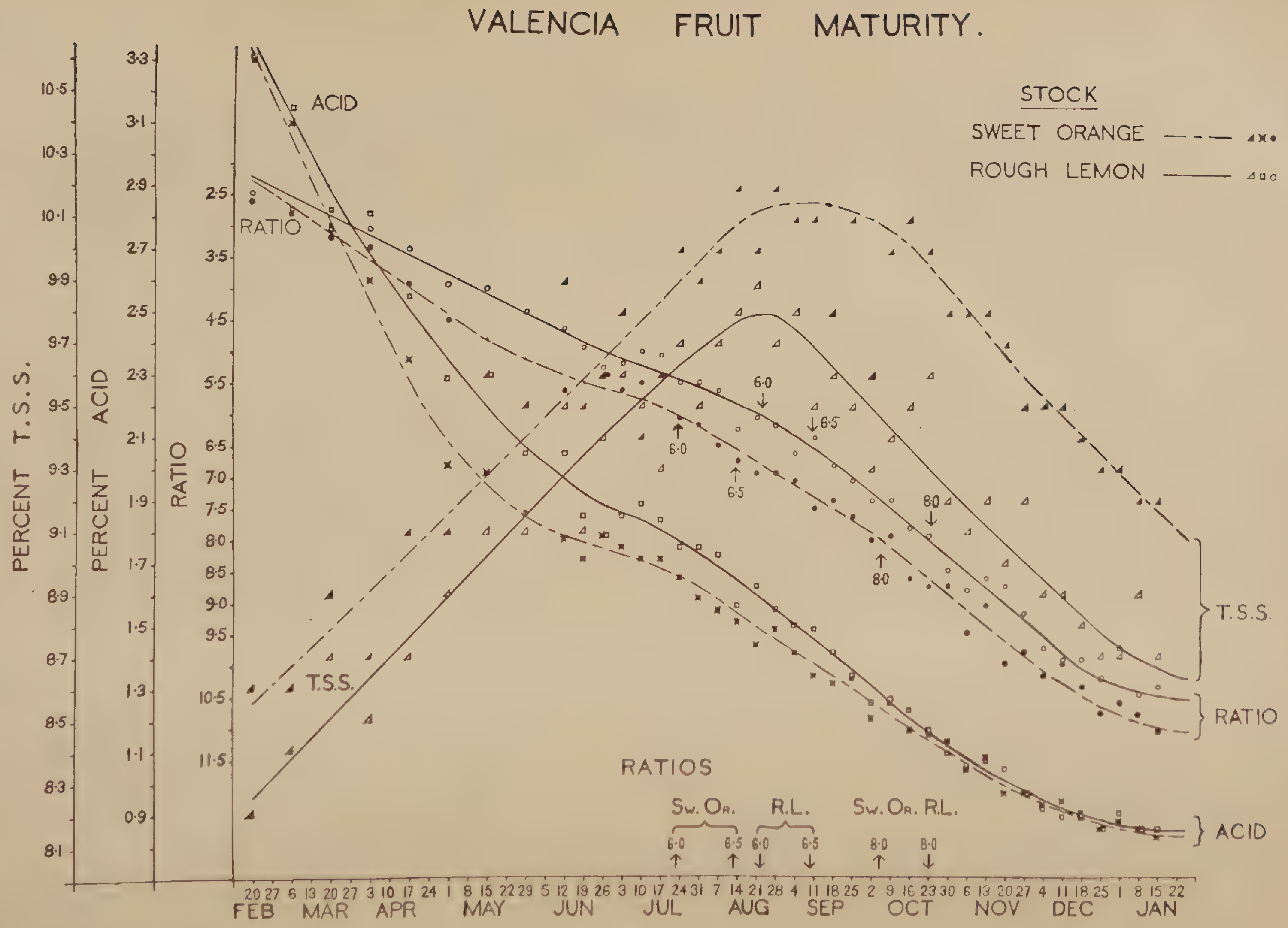

Fig. 4.

Graphical representation of changes which occurred over an eleven-months period from immaturity to over-maturity in the total soluble solids content, acid content, and ratio of the two in the juice of Valencia oranges from trees on sweet orange and rough lemon stocks.

Citric acid content.

The content of citric acid is the other important factor determining fruit internal quality. The curves for the percentage acid in the juice (Fig. 4) show that the initial high values, about 3·3 per cent., found in immature fruit in February fell rapidly to a break in rate of decrease in May-June, giving values of about 1·87 per cent. coincident with the main colour change of the fruit, and thereafter decreased more gradually to a value of about 0·9 per cent. at over-maturity in January. The acid content determines the start of harvesting in commercial citrus-growing, for until it has diminished to such a figure that the fruit is not sour to the taste, the fruit cannot be regarded as being of even passable eating quality. The author (1949a) has shown that with Valencia oranges from trees on rough lemon and sweet orange stocks, significant differences in acid content of the juice cannot be taken as a physiological characteristic, and it is concluded from the curves in Fig. 4 that the acid content of fruit from trees on the two stocks does not differ during the growth period from immaturity to over-maturity.

The ratio of total soluble solids to acid.

In the juice of citrus this ratio is used in Government Regulations (1948) with qualifying requirements of minimum juice, T.S.S., and acid values, as a gauge of degree of maturity of the fruit ; for Valencias a ratio of 6 : 1 (in California it is 8 : 1) must be reached before the fruit may be packed for export. It is seen in Fig. 4 that the ratio values lie practically on a straight line during the ripening of the fruit, paralleling those of acid from June onward. A relatively small change in acid value had a marked influence on the ratio in relation to changes which occurred in T.S.S. values. Fig. 4 shows that in the year 1939 in the orchard under observation the ratio of 6 : 1 was reached by the third week in August for trees on rough lemon stock, and four weeks earlier for trees on sweet orange stock. The fruit from trees on rough lemon had attained its maximum T.S.S. content by the time the ratio was 6 : 1, and that particular fruit might have been considered to be in its best eating condition, whereas fruit from trees on sweet stock did not attain its maximum T.S.S. content till four weeks after its 6 : 1 ratio had been reached, by which time it had a ratio of 6·8 : 1. Fruit from trees on sweet stock maintained its maximum T.S.S. content till the first week in October, and then had a ratio of 8 : 1, and was of excellent eating quality, far superior to fruit from trees on rough lemon also having an 8 : 1 ratio, even though the latter was three weeks later, due to the early decrease in T.S.S. If the Government Regulations required a ratio of 6·5 : 1, an increase of only 0·5, it would have meant that the start of harvesting of the crop would have been three weeks later ; this point is of the greatest practical importance in connection with the regulation of supply of fruit to both local and export markets.

GENERAL.

Time of blossoming of any particular variety of citrus may vary by several weeks from year to year and in different areas, being influenced everywhere by climatic conditions, and for specific trees or orchards especially by the moisture content of the soil in the root zone. This variation in itself results in variation in the time at which the fruit matures, but seasonal variations in climatic conditions pertaining during the growth and development of the fruit also have a marked influence in determining the time taken for the fruit to mature, as well as on its size, rind thickness, juice content, percentage acid, percentage T.S.S., and the ratio of the latter two factors. The average values from 125 fruit-quality tests of Valencia oranges made yearly over a ten-year period on the same date each year with fruit from the same trees (including the trees from which the fruit used in this work was obtained) showed with fruit of 176 pack-size the following maxima and minima in different years: rind thickness ·55 cm. to ·48 cm., juice 55 per cent. to 49·5 per cent., T.S.S. 10·3 per cent. to 8·9 per cent., acid 1·44 per cent. to 1·16 per cent., and ratio of T.S.S. to acid 8·6 : 1 to 6·5 : 1. The average weight per fruit for the total crop varied from ·48 lb. to ·40 lb.

Thus, while the observations in this paper show the course of development of the Valencia orange as regards growth and fruit quality in one year in the Eastern Transvaal, absolute values will vary from year to year and from place to place. It follows that, if a truer gauge of fruit maturity is desired on which time and stage of picking is to be based, more work is needed. Modified studies, along the lines of this investigation but over a period of years, should be undertaken for all commercial citrus varieties in the main citrus-growing areas.

SUMMARY.

Fruit-growth and internal changes in fruit quality have been studied from immaturity to over-maturity in Valencia oranges developing on trees on sweet orange and rough lemon stocks. The trees were growing under mild subtropical conditions with summer rainfall.

Weekly measurements of fruit diameter were made over a period of twelve months, and by the use of a dendrograph the daily fluctuations in diameter of an individual fruit were recorded over a period of nine months.

Examination of ten years' records showed that, in general, small fruit is associated with heavy crops and vice versa, but in three of the years some dominant seasonal factor upset the correlation.

Young trees on sweet orange stock were shown to produce slightly smaller fruit than did trees of the same age on rough lemon stock, an initial difference in size being maintained throughout development.

The critical growth-period of the fruit is the first seven months after setting. The effect of a water deficit in the root zone during that period was a reduction in size of fruit at maturity by more than one pack-size. After the critical period, water deficits brought about a temporary cessation of increase in size, or even a decrease, but had no permanent effect after recovery following replenishment of soil moisture.

Dendrographic records showed marked diurnal changes in fruit diameter during the first seven months after setting, the size being at a maximum early in the morning and decreasing towards mid-afternoon. After the critical period had passed, diurnal changes gradually ceased.

Rind thickness showed a direct correlation with fruit diameter after the small immature stage of the fruit was passed, the ultimate thickness being determined at an early stage in fruit development. Fruit from trees on sweet orange stock had initially thinner rind than fruit from trees on rough lemon stock and maintained the difference right through to over-maturity.

Fruit from trees on sweet orange stock had a higher juice percentage during the maturity period than fruit from trees on rough lemon. A juice content near the maximum was attained and held after slowing-up of growth of fruit and colour changes in the rind.

Fruit from trees on sweet orange stock had a higher initial content of total soluble solids (T.S.S.) in the juice than fruit from trees on rough lemon ; this difference increased at maturity. The T.S.S. of the juice increased from immaturity to maturity and then decreased to over-maturity, but the start of decrease was some weeks later on sweet orange than on rough lemon.

The acid content of the juice decreased from immaturity to over-maturity, with a break in rate when the fruit approached full size and the colour changes started.

The ratio of T.S.S. to acid in the juice of the fruit was found to follow mainly the changes in acid content, especially just before and during maturity. Due to its higher T.S.S. content, juice of fruit from trees on sweet orange stock had a higher ratio throughout development, reaching any given ratio some four weeks earlier than did the juice of fruit from trees on rough lemon stock.

The desirability of similar studies with all commercial varieties of citrus in various citrus-growing areas having different climatic conditions is noted.

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STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES

I. FIELD TRIALS USING VARIOUS SYNTHETIC COMPOUNDS FOR THE SETTING OF OUTDOOR TOMATOES

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THE first report that tomato ovaries can develop into seedless fruit as a result of application of synthetic growth substances was published by Gustafson (1936). In this and other investigations carried out during the next few years (Schroeder (1937); Howlett (1939, 1941, 1942)), β-indolylacetic acid and other compounds which had proved active in promoting the rooting of cuttings were employed. Zimmerman and Hitchcock (1941) were the first to demonstrate that β-naphthoxyacetic acid was effective when used as a fruit-setting spray on tomatoes. Subsequent work by these investigators at the Boyce Thompson Institute (Zimmerman and Hitchcock (1942, 1944, 1944a)) has revealed a large number of synthetic compounds which are active in causing fruit development and there is no doubt that many new ones remain to be discovered.

Under certain conditions, fruit-setting compounds can be of great value in supplementing the natural set of fruit and a number of preparations containing β-naphthoxyacetic acid for use on the tomato are now available commercially. The tomato plant differs greatly in its degree of response to different fruit-setting compounds. Frequently, undesirable effects such as the development of hollow mis-shapen fruits, epinasty of leaves and stem and growth of thin elongated leaves may follow the use of these materials, especially when they are applied at too high a concentration (Zimmerman and Hitchcock (1944a); Swarbrick (1944)). There is also evidence that the flavour of the resulting fruit may be dependent to some extent on the fruit-setting compound employed. The type of chemical required is, therefore, one which, when sprayed at the recommended concentration, produces a high set of good quality fruit with no undesirable effects on the plant. Further, since such effects are liable to arise with any of these materials when applied in excess, a wide margin is desirable between the optimum concentration and that at which detrimental effects may be produced.

Satisfactory results have been obtained using sprays containing β-naphthoxyacetic acid or one of its salts both in trials and under commercial conditions. When this material is applied to the open flower truss at a concentration of some 40-60 p.p.m. an excellent set of fruit is obtained and undesirable effects are usually negligible. Nevertheless there is always the possibility that even better fruit-setting compounds exist. To establish the superiority of such a compound or mixture of compounds, trials carried out under different conditions are necessary. Also, the quality as well as the yield of fruit must be assessed.

In America some of the more promising of Zimmerman and Hitchcock's compounds have been included in fruit-setting trials, most of which have been carried out on greenhouse tomatoes. Thus Roberts and Struckmeyer (1944) found that α-(2:4-dichlorophenoxy)-propionic acid was as effective in setting tomatoes as β-naphthoxyacetic acid at the concentrations used. Sprays containing α-(2-chlorophenoxy)-propionic acid and 2:4-dichlorophenoxyacetic acid, however, though effective, produced damage. The latter substance was found by Murneek *et al.* (1944) to be a most active material though capable of producing hollow and misshapen fruit. Similar results were obtained with *p*-chlorophenoxyacetic acid sprays. Howlett and Marth (1946), in an experiment where spraying was carried out every five days so that each truss received approximately three applications, found a mixture of β-naphthoxyacetic acid and β-indolylbutyric acid superior to the former substance alone. Murneek (1947) carried out investigations with various growth substances on tomatoes both in the greenhouse and in the open. He found that *p*-chlorophenoxyacetic acid had the greatest effect in increasing fruit-set and size but under certain conditions gave " puffy " fruit. For this reason β-naphthoxyacetic acid was preferred. Other compounds investigated were α-(2-chlorophenoxy)-propionic, α-(2:4:5-trichlorophenoxy)-propionic and 2:5-dichlorobenzoic acids, the latter material not being very effective at the concentration employed. Under greenhouse conditions, spraying the whole plant using a reduced concentration of active substance was found to be more effective than spraying the flower truss only (see also Murneek *et al.* (1944)).

On outdoor plants in America, Zimmerman and Hitchcock (1944a) were fairly successful in producing seedless fruits during cold wet weather when natural setting was poor. During the warmer growing season, however, in no case was an increase in the yield from treated over control plants noted. In agreement with this, Murneek (1947) found no significant increases in yields from outdoor plants following applications of β-naphthoxyacetic or *p*-chlorophenoxyacetic acids. Wittwer *et al.* (1948) discuss the factors responsible for poor natural setting of outdoor tomatoes. Important among these is low night temperatures (Went and Cosper (1945), Smith and Cochran (1935)), poor setting usually resulting with temperatures below 55° F. In Michigan where low night temperatures are frequently experienced, Wittwer *et al.* (1948) obtained significant increases in yield, particularly of early fruit, by spraying the first truss of outdoor plants with *p*-chlorophenoxyacetic acid at 25 p.p.m. Although weather conditions in upper Michigan and other Northern States may not favour optimum natural setting on outdoor plants so that a fruit-setting spray may be of value, this does not apply in other parts of the country and, in general, it would appear that natural setting of outdoor tomatoes during the normal growing season in the United States presents no problems. In this country, however, the climatic conditions are such that it is often difficult to get a good natural set of tomatoes on the bottom truss of outdoor plants. In such circumstances, a good response to fruit-setting sprays is usually achieved. Swarbrick (1944) was the first to demonstrate the value of β-naphthoxyacetic acid as a fruit-setting spray for tomatoes under English conditions. There is very little published work, however, on the results of outdoor trials carried out in this country.

For a number of years the writer has been making investigations with growth-regulating substances with particular reference to the relationship between chemical structure and physiological action on plants, a field in which Zimmerman and Hitchcock and Veldstra have already made notable contributions. In the course of the Wye investigations some eighty compounds have been synthesized and examined for growth-promoting activity by various means including the property of stimulating parthenocarpic development of tomato ovaries. Some of the compounds showing promise in the latter tests have been included in the field trials now reported.

LAY-OUT OF TRIALS.

Large scale trials to compare the effectiveness of different chemicals in setting the bottom truss of outdoor tomatoes were carried out in 1947 and 1948 at Nackington Farms, Canterbury, and at Wye College in 1948. In each case four treatments were included together with control unsprayed plants. The lay-out in the three trials was identical except in regard to the number of plants in the experimental rows; these details are given below.

Each experimental block consisted of 19 rows of plants. Alternate rows only were used, however, the intervening rows serving to separate the experimental rows and thus prevent drift of spray from one row to another. Each experimental row was further divided into two half-rows with baffle plants between and at each end. Each of the four sprays was used on four half-rows and there were also four half-rows of control plants in each experiment. Row positions were randomized over the experimental block.

METHODS.

Spraying was carried out on the bottom truss only, this being the most valuable commercially and yet the most difficult to set naturally on outdoor plants in this country. A portable pneumatic-type sprayer holding some 1½ pints of solution was employed. The spray was directed well into the truss and behind the calyces and although individual treatment of each flower was not attempted, good wetting of the whole truss was usually achieved within 5-10 seconds.

In each trial, spraying was performed when it was considered that a maximum number of flowers on the bottom trusses were open; at this stage a few naturally set fruits were usually encountered on each truss. The whole spraying programme in each trial was carried out within a few hours.

The experimental rows were given the same cultivation, " blight " protection and general treatment as was received by the remainder of the field, the only difference being in the spraying and picking of the crop. Fortunately the experimental rows remained practically disease-free during all three trials. In a few cases, however, single diseased plants had to be removed and where this was done before cropping started an appropriate correction to the row yields has been made. In the Canterbury 1947 experiment a small number of plants (9 out of 1,200) which had to be replanted a week after the main planting, received a set-back and formed puny plants with a smaller fruit yield.

Fruits from the bottom trusses of the experimental plants were picked on the same days at the orange-green or orange stage and row weights were recorded. At the last pickings a small number of green fruits were usually included in the weights from each row. Random samples of fruit from the different treatments were examined periodically during the picking season for quality and flavour and, towards the end of the trials, larger quantities of fruit were set aside for more detailed examination.

CANTERBURY TRIAL, 1947.

Each of the four sprays was applied to the bottom trusses of four 60-plant rows in a four-acre field of outdoor tomatoes (var. " Harbinger ") at Nackington Farms The four control rows received no treatment. Spraying was carried out under good weather conditions on 1st July using the following :

DCA.	Eutectic mixture of 2:4- and 2:6-dichlorophenoxyacetic acids* (4 p.p.m. of acids as sodium salts).
BNA.	β-naphthoxyacetic acid (60 p.p.m. of acid as sodium salt).
OCP.	α-(2-chlorophenoxy)-propionic acid (40 p.p.m. of acid as sodium salt).
BNP.	α-(2-naphthoxy)-propionic acid (100 p.p.m. of acid as sodium salt).

Each spray contained 0·004 per cent. of a proprietary wetting agent of the sodium secondary alcohol sulphate type.

Weather had been dry during the previous fortnight and from 2 to 5 fruits per truss had set naturally at the time of spraying.

RESULTS.

(*a*) *Yield.* Fruit ready for picking was removed from all experimental rows on the same days and weights were recorded. Total yields are given in Table I and the progressive yields in Fig. 1. Statistical analysis of these results reveals no significant differences between the four spray treatments though the mean yield from the treated rows (1,285 oz. per 60-plant row) was significantly greater than that from the controls (994 oz. per 60-plant row) at the 1 in 20 level.

TABLE I.

Total yields (in ounces) of fruit from bottom trusses (Canterbury 1947).

Treatment.	60-plant row.				Total (240 plants) oz.
	I.	II.	III.	IV.	
DCA ..	1,366	1,232	1,350	1,300	5,248
BNA ..	1,382	1,379	1,162	1,281	5,204
OCP ..	1,440	1,325	1,125	1,176	5,066
BNP ..	1,364	1,228	1,307	1,143	5,042
Control ..	926	946	1,015	1,089	3,976

(*b*) *Earliness.* Fig. 1 shows that there are only slight differences in the capacities of the different sprays to produce early fruit although, over the first fortnight of picking, plants which had received the BNA spray gave a somewhat lower yield than did those which had received the other treatments.

(*c*) *Fruit size and quality.* Evenness in size of fruit was a marked characteristic of all sprayed rows compared with the controls though the DCA spray tended to produce larger fruit than did the other treatments. This spray gave a small number of hollow fruits amongst the early pickings and, towards the end of the truss, up to 70 per cent. of the fruit was so affected. Such fruit also tended to be badly coloured, streaked with dark green and definitely of a poor selling quality. Hollow fruit was also found in rows which had received other treatments though not to any large extent at any time. The BNP plants, however, bore very few such fruits, the shape and size of nearly all tomatoes from this treatment being most satisfactory. Even control plants showed a few hollow fruits at the end of the truss and this feature was also found among fruit from the rest of the field outside the experiment. It is possible that this was associated with the abnormally dry weather experienced.

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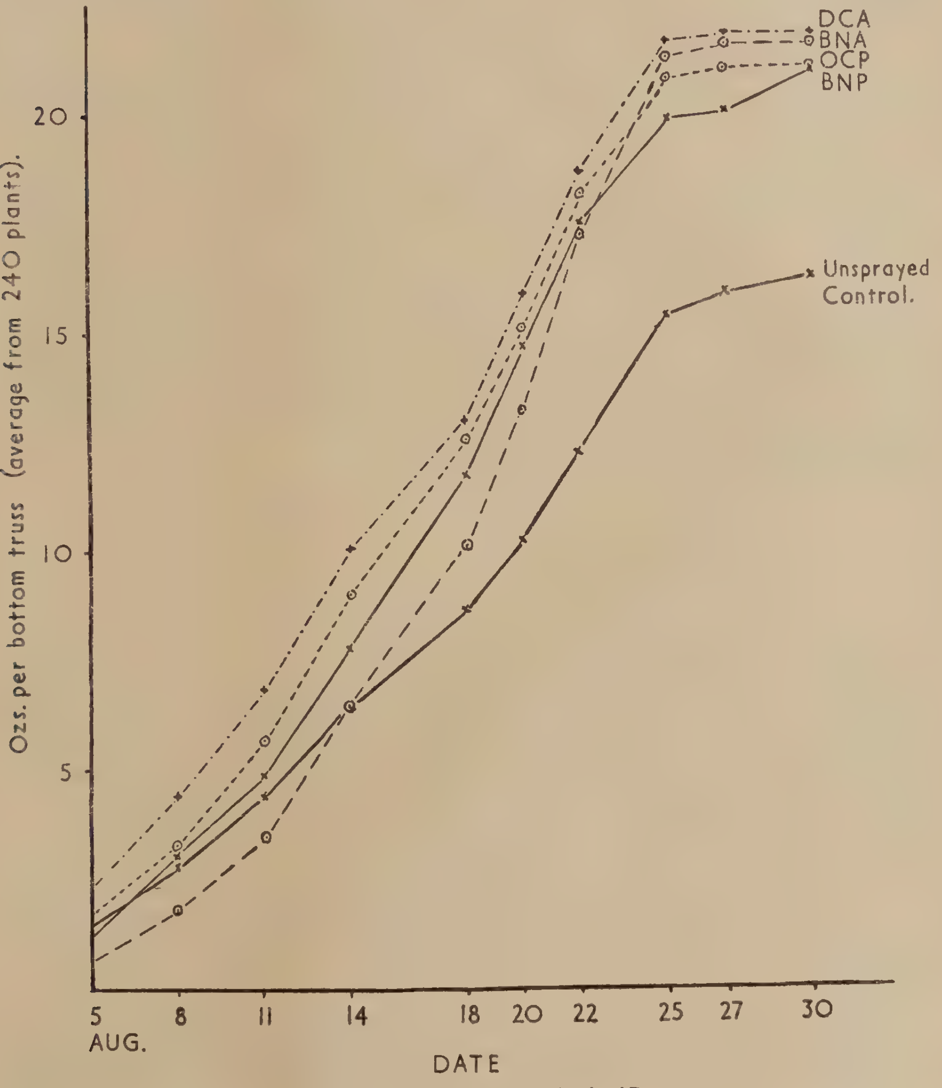
* See note at end of this paper.



CANTERBURY TRIAL 1947

FIG. 1.

Other internal differences in the fruit from the different treatments were also noted. DCA fruit had thick flesh and tough skin, especially towards the end of the life of the truss ; other sprayed rows yielded fruit with normal or slightly thinner flesh and normal skins. In the early and middle ripening stages, tomatoes from the BNA, OCP and BNP sprayed rows appeared to have watery pulp as opposed to the gelatinous pulp of the control fruit. This effect disappeared towards the end fruit of the trusses.

Two- and three-rayed fruit occurred impartially among all rows.

Some pointed fruits were found in all treated rows but not on control plants. The numbers of such fruit were small, however, except in the rows which had received the DCA spray. Here the effect was much more marked ; for example, up to 40 per cent. were so affected in the 25th August picking from these plants.

On 25th August, 6 lb. lots of ripe fruit were taken from the pickings of control and treated rows and a panel of seven persons carried out tasting tests in the laboratory.

The procedure was as follows. Samples of tomatoes from each lot were cut downwards into quarters and each quarter was halved by cutting across. The unnamed samples so prepared were tasted by each observer. Some 2-4 pieces were eaten before marks (out of 10) were awarded, and often a sample was tasted again to settle a fine difference in flavour between one sample and another. Each observer judged independently and was unaware of the marks awarded by the others ; no discussion took place during the test.

Scoring was as follows (maximum points 70) :

Control	64
OCP	58
BNA	55
BNP	51
DCA	43

The flavour of control fruit was unanimously agreed to be superior to the rather insipid flavour of the parthenocarpic fruit.

The OCP and DCA fruit were found to possess tougher skins than the other fruit, the DCA sample being particularly bad in this respect. Many hollow fruits were found in the DCA sample but all other tomatoes were free from this defect.

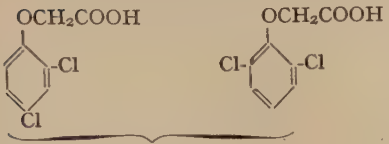
1948 EXPERIMENTS.

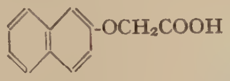
In these two trials, carried out at Canterbury and at Wye, the following spray treatments were employed :

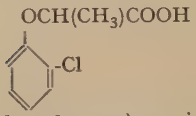
OCP	α-(2-chlorophenoxy)-propionic acid (40 p.p.m. of acid as methylglucamine salt).
BNA	β-naphthoxyacetic acid (60 p.p.m. of acid as methylglucamine salt).
BNP	α-(2-naphthoxy)-propionic acid (100 p.p.m. of acid as methylglucamine salt).
Mixture	β-naphthoxyacetic acid (40 p.p.m. of acid as methylglucamine salt) and *p*-chlorophenoxyacetic acid (10 p.p.m. of acid as methylglucamine salt).

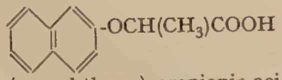
Each spray also contained 0·004 per cent. wetting agent.

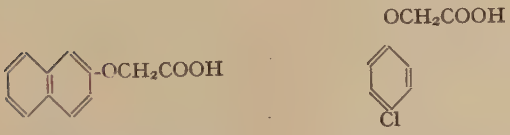
The following are the formulae of substances used in these trials.

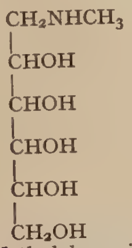

Dichlorophenoxyacetic acids
DCA


β-naphthoxyacetic acid
BNA


α-(2-chlorophenoxy)-propionic acid.
OCP


α-(2-naphthoxy)-propionic acid.
BNP


β-naphthoxyacetic acid. *p*-chlorophenoxyacetic acid.
Mixture



$$CH_2NHCH_3-CHOH-CHOH-CHOH-CHOH-CH_2OH$$

Methylglucamine

CANTERBURY TRIAL 1948.

The lay-out and numbers of plants were exactly the same as in the 1947 trial. Sprays were applied to bottom trusses during the afternoon of the 1st July in dull but fine weather. The plants (var. " Harbinger ") were sturdy and short-jointed with up to two fruits developing on each bottom truss.

RESULTS.

(*a*) *Yield.* Total and progressive totals are shown in Table II and Fig. 2. Statistical analysis of these results revealed no significant differences in the yields from the four spray treatments though the mean crop from the treated rows (790·5 oz. per 60-plant row) was significantly greater than that from the controls (573·0 oz. per 60-plant row) at the 1 in 20 level.

TABLE II.

Total yields (in ounces) of fruit from bottom trusses (Canterbury, 1948).

Treatment.	60-plant row. I.	II.	III.	IV.	Total (240 plants) oz.
OCP ..	676	866	695	762	2,999
BNA ..	606	773	757	822	2,958
BNP ..	837	836	785	591	3,049
Mixture ..	940	815	967	921	3,643
Control ..	505	509	674	604	2,292

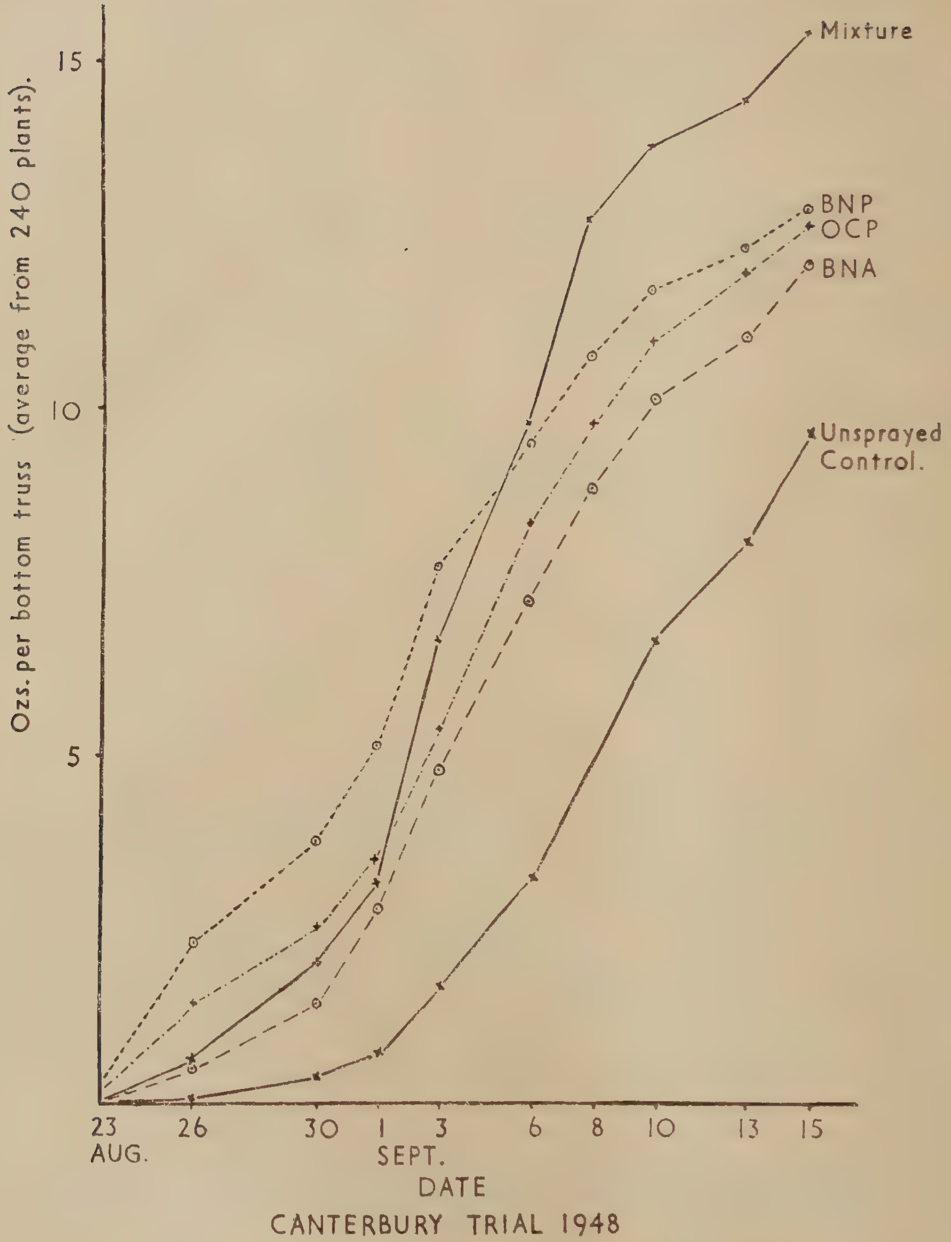


FIG. 2.

(*b*) *Earliness.* All sprayed plants produced earlier fruit than the controls (Fig. 2) and the BNP spray was somewhat better in this respect than the other treatments.

(*c*) *Fruit size and quality.* Sprayed rows yielded fruit of even size, the average number per pound over the whole picking period being OCP 6·2, BNA 7·1, BNP 8·5, Mixture 7·3 and Control 11·2. The control rows yielded some 5-6 times as many small fruits as the treated rows. Fruit from the rows sprayed with Mixture was considered by the recorder to be better in general appearance when viewed in quantity than that from other treated or control rows.

Only few pointed fruits were found throughout the experimental block except in the OCP rows ; even here the feature, though common, was only slight.

Samples of fruit from each picking were cut for examination of the contents. Only few hollow fruits were found except towards the end of the period when most of the fruit was so affected to a slight extent. The feature was not sufficiently marked to affect appreciably the commercial value of the fruit. On several occasions it was noted that tomatoes from the BNP rows appeared to have a more pulpy centre than the other treated fruit.

A few cracked fruits appeared in all control and treated rows and in rows outside the experiment, an effect probably related to weather conditions. No distinct differences in toughness of skin of fruit from the different treatments were recorded.

Uneven ripening was common amongst all early fruit but this became normal towards the middle of the period except for a few fruits in the BNP rows.

On 14th September, 6 lb.-samples of fruit from control and treated rows were taken for detailed examination. Seven persons took part, each awarding marks out of 10 for a particular sample in each test. The results are given, together with those from the 1948 Wye trial, in Table IV.

WYE TRIAL, 1948.

The plants (var. " Harbinger ") used in this trial formed part of a half-acre block of outdoor tomatoes in the Wye College Horticultural Department. The same lay-out was adopted as in the two trials already described, except that each experimental row consisted of 48 and not 60 plants.

Spraying was carried out during the afternoon of 29th July, a typical bottom truss then consisting of 0-2 fruits set, 3-7 unfertilized ovaries, and 1-3 flowers and buds. The plants were strong and healthy but some 3-4 weeks later than those in the Canterbury experiment.

RESULTS.

(*a*) *Yield.* The numbers of fruits from each treated and control row as well as the yields were recorded in all pickings until 23rd September. On this date, the numbers of fruits remaining on the trusses were counted. These figures, together with those representing picked fruit, gave the total numbers set in control and treated rows. The results are shown in Table III in which total yields are also given. Progressive yields are shown in Fig. 3.

Statistical analysis of the results shows that the BNA treatment gave crop weights which were significantly lower (at the 1 in 20 level) than those given by the OCP and Mixture treatments. The mean yield with the spraying treatments (642·2 oz. per 48-plant row) was significantly higher than in the controls (161·0 oz. per 48-plant row) at the 1 in 100 level.

(*b*) *Earliness.* There was practically no difference between the treatments in respect of production of early fruit (Fig. 3).

(*c*) *Fruit size and quality.* Extremely poor natural setting conditions were experienced during the period of this trial and many blossoms in the control rows

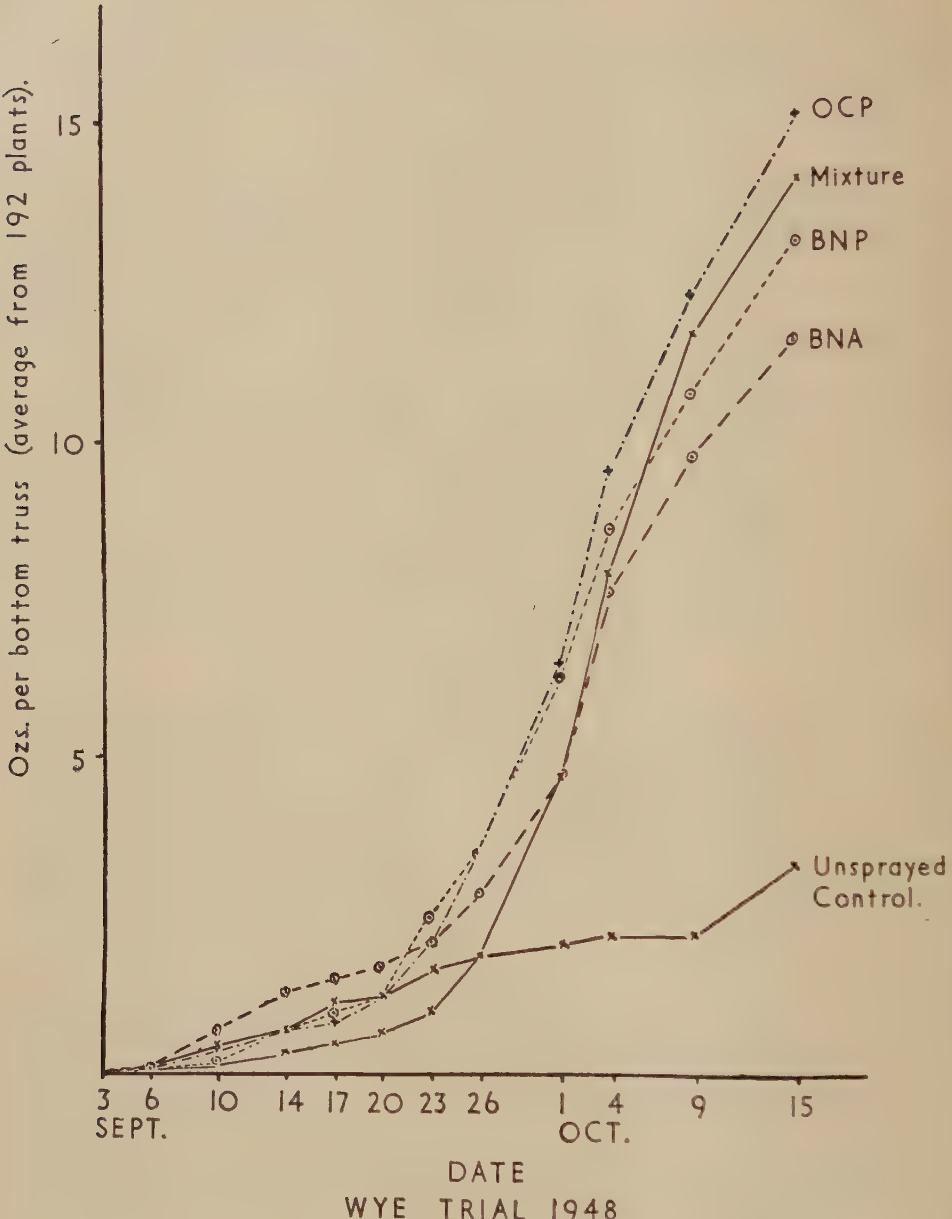


Fig. 3.

either abscissed or gave small "dry-set" fruit. Some "dry-sets", arising from flowers which were in the bud stage at the time of spraying, were also found in the treated rows. In this trial, such fruit was not picked and is therefore not included in the total yields though it is included in the total numbers of fruits (Table III).

Pointed and hollow fruits were common from the OCP rows though the features

TABLE III.

Total yields from bottom trusses and numbers of fruit set (Wye, 1948).

Treatment.	Wt. per 48-plant row (oz.). I.	II.	III.	IV.	Total Wt. (192 plants) oz.	Total No. of fruit set (192 plants).
OCP ..	687	764	693	745	2,889	1,947
BNA ..	639	416	507	652	2,214	1,785
BNP ..	657	562	667	615	2,501	1,901
Mixture	629	658	725	670	2,682	2,030
Control ..	143	179	140	182	644	787

were not marked. Other fruit was almost completely free from these defects. Fruit ripened evenly except in the OCP rows where there was some slight tendency to green streaking. In samples tasted from the various pickings it was recorded that the OCP fruit tended to possess a tougher skin than did tomatoes from other treated rows. When viewed in bulk, fruit from control rows and rows which had received Mixture was superior to the rest.

On 16th September all plants were stripped of fruit of marketable size and the picking included green fruits; 3 lb.-samples of the riper fruit were taken for assessment of quality and flavour by a panel of nine persons each of whom awarded marks out of 10 for each sample in each test (Table IV).

TABLE IV.

Assessment of fruit quality, 1948 trials.

Feature.	Site.	Maximum marks.	OCP.	BNA.	BNP.	Mixture.	Control.
Shape	Canterbury	70	38	43	59	42	70
	Wye	90	41	68	57	75	77
Even ripening	Canterbury	70	48	39	38	36	62
	Wye	90	48	60	64	72	74
Interior appearance	Canterbury	70	42	50	52	52	63
	Wye	90	53	66	58	72	62
Flavour	Canterbury	70	45	48	48	46	54
	Wye	90	58	63	57	64	80

DISCUSSION.

Although under American conditions the effect of hormone sprays on the yield of outdoor tomatoes is usually small, due to the high percentage of natural setting, the trials here reported have clearly demonstrated that in this country crop yields may be substantially increased by such treatment. Even here, however, climatic conditions during the period when normal fertilization occurs are of great importance in relation to the final yield of crop from untreated plants. It is uncertain whether under dull cool conditions, especially at night, an inadequate amount of pollen or pollen of low viability is produced. Although the grower speaks of "good" and

" poor " setting years it would seem that it is mainly the weather conditions just before and during the period when the ovaries are receptive to pollen which determines the amount of natural set. The fact is well brought out in the two 1948 trials described in this paper. Although 1948 was generally considered to be a poor setting year, there were in fact about ten days of hot weather with high night temperatures around the time when the flowers on the bottom trusses at Canterbury were ready for fertilization, a condition which enabled a reasonable yield from the control plants in this trial to be harvested. On the Wye plants, however, which were some three weeks behind those at Canterbury, flowers on the bottom trusses were only in the bud stage during those ten days and by the time they had opened and were receptive, poor setting conditions prevailed. This explains why the yield from control plants at Wye (3½ oz. per bottom truss) was extremely low. It is under such conditions, where poor natural setting results, that spraying with growth substances produces a spectacular increase in yield (Table III ; Fig. 3). Instead of a truss consisting of a small number of marketable fruits together with a few useless " dry-set " tomatoes, the other flowers having abscissed (Plate I, Fig. 1), a full truss of fruits of uniform size can be harvested. Plate I, Fig 2 shows an exceptionally fine truss produced by spraying with BNP the bottom truss of a plant close to that shown in Plate I, Fig. 1.

In all three trials here discussed the mean yield of fruit from treated rows was significantly higher than that from control rows. No significant differences in yields from the four treatments in the 1947 trials were found though all four sprays increased the yield to a useful extent when comparison was made with the yield from control plants. The DCA fruit, however, was of inferior market value having a high proportion of hollow and pointed tomatoes. The skin of this fruit also tended to be tougher than that from control and other treated plants and the flavour was inferior. On the other hand, the BNA, OCP and BNP fruit was considered to be of sufficiently good quality to justify the inclusion of these treatments in the 1948 trials. Accordingly, the DCA treatment was replaced in these experiments by a mixture of β-naphthoxyacetic acid and *p*-chlorophenoxyacetic acid (Mixture). Our small scale experiments with these two materials had shown that whereas *p*-chlorophenoxyacetic acid used alone was highly active in setting tomatoes, this compound tended to produce hollow and pointed fruit. Various sprays containing this material, in combination with β-naphthoxyacetic acid, however, were found here not only to be highly active in setting tomatoes but also to produce fruit of good quality.

All compounds in 1948 were used as their methylglucamine salts. This organic base gives rise to salts of very high water solubility, indeed several solid preparations were made containing the salt and wetter which readily dissolved in water to give a spray ready for use. Preliminary experiments had shown that methylglucamine salts were in no way inferior to sodium salts for setting tomatoes nor were they in any way phytotoxic at the concentrations required.

In the 1948 Canterbury experiment again there was no significant difference in the yield from the four spray treatments. In the Wye trial, however, both the Mixture and the OCP treatments gave significantly greater yields of fruit than did the BNA spray. In quality the fruit from the Mixture was considered satisfactory in both trials. The tomatoes were well filled with gelatinous material and there were no hollow regions. Their flavour was in no way inferior to that of fruit from other treatments though it was not so acceptable as that of the normally seeded fruit.

Tomatoes from the OCP rows were of somewhat lower quality than were those from other treatments. Some pointed fruits were found and there were indications of more uneven ripening and tougher skins. None of these defects was serious, however, and it is doubtful whether they lowered the market value appreciably.

The BNA sprays led to the production of good quality fruits. Salts of β-naphthoxyacetic acid (BNA) form the active material in a number of proprietary fruit-setting sprays on the British market. Such products, when used according to the makers' instructions, have usually given excellent results. As far as the writer is aware, none of these products when diluted for use contains more than the equivalent of 60 p.p.m. of β-naphthoxyacetic acid; some in fact contain only 40 p.p.m. It would therefore appear unlikely that a better setting performance could have been obtained using a proprietary spray containing β-naphthoxyacetic acid than was achieved with the BNA treatment in the trials here reported.

The BNP spray in all these trials produced a satisfactory set of good quality fruit and in the writer's opinion is at least as good as the BNA spray in these respects as a treatment for outdoor tomatoes. With glasshouse tomatoes, however, BNP at 100 p.p.m. has been found to cause some plant distortion especially after leaves in the neighbourhood of flower trusses had received some of the spray. This undesirable effect was apparent on the day following treatment and although the plants completely recovered within 3-4 days, the fact that such distortion may result provides a decided objection to the use of the BNP spray on indoor plants. No such effect has been observed in our experience with BNP on outdoor plants, nor were any such undesirable responses shown following any treatment in these trials.

The experiments here reported reveal the difficulties in assessing the value of different compounds for setting outdoor tomatoes. In spite of the fact that large numbers of plants were used, there was in some cases marked variation in the yields from replicated rows. Nevertheless, the value of fruit-setting sprays in increasing the yield of outdoor tomatoes has been clearly demonstrated in all three trials. Since all compounds used were highly effective in this respect, the assessment of fruit quality became the important factor in deciding their relative merits. On this basis the DCA and OCP treatments were less satisfactory than the rest. In one trial the Mixture was more effective in setting than the BNA and produced good quality fruit. It would appear that sprays containing both β-naphthoxyacetic acid and *p*-chlorophenoxyacetic acid warrant further investigation.

SUMMARY.

The results of three large-scale trials are presented in which the sodium or methylglucamine salts of various synthetic growth-regulating substances were used as fruit-setting sprays on the bottom trusses of outdoor tomato plants during the seasons 1947 and 1948. Crop yields and estimates of fruit quality were used to assess the relative value of the different treatments.

β-naphthoxyacetic acid, the active constituent of proprietary sprays, was included in all trials.

Each treatment employed led to a significant increase in yield of fruit over that obtained from unsprayed control plants, the mean increase in yield due to

treatment being 29·5 per cent., 37·9 per cent., and 299·3 per cent. in the three trials. Weather conditions during the period at which normal fertilization could take place largely influenced the increase in yield obtained by spray treatment.

Sprays containing dichlorophenoxyacetic acids at 4 p.p.m. and α-(2-chlorophenoxy)-propionic acid at 50 p.p.m. though effecting good sets, produced fruit of inferior quality. β-naphthoxyacetic and α-(2-naphthoxy)-propionic acids at 60 and 100 p.p.m. respectively gave satisfactory results.

A mixture of β-naphthoxyacetic acid at 40 p.p.m. and *p*-chlorophenoxyacetic acid at 10 p.p.m. gave an excellent set of good quality fruit and was considered the best of the sprays examined. Further investigations with this and similar mixtures as fruit-setting sprays for tomatoes are warranted.

ACKNOWLEDGMENTS.

The writer gratefully acknowledges the assistance of Messrs. R. L. O. Jackson, B.Sc., R. E. James-Robertson, F. W. M. Llewellyn, B.Sc., P. K. Isaac, B.Sc., Miss E. M. Barker and Miss R. Smart, B.Sc., who were responsible for recording these trials. He also thanks Mr. Hugh Finn and Professor H. W. Miles for providing facilities at Canterbury and Wye respectively, Dr. H. J. Barber for supplying the methylglucamine used in the 1948 trials, and Dr. J. Taylor of East Malling Research Station who carried out the statistical analyses.

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PLATE I.

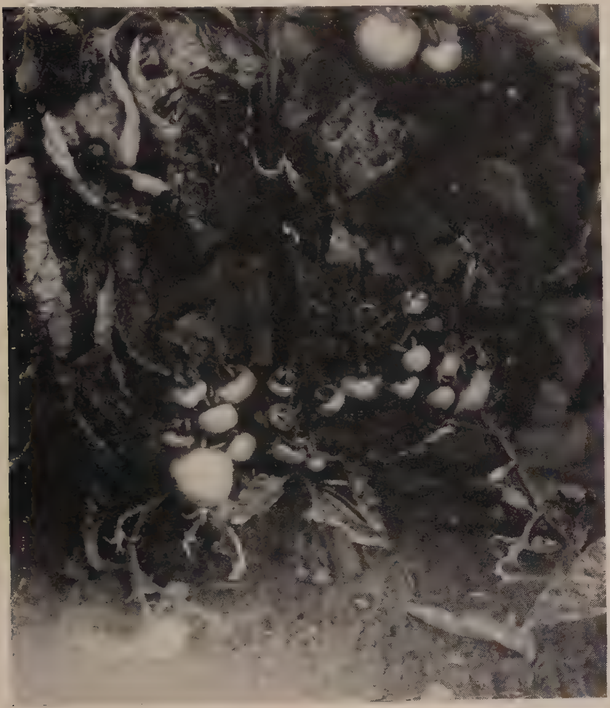

FIG. 1.

Untreated bottom truss of outdoor tomato plant.

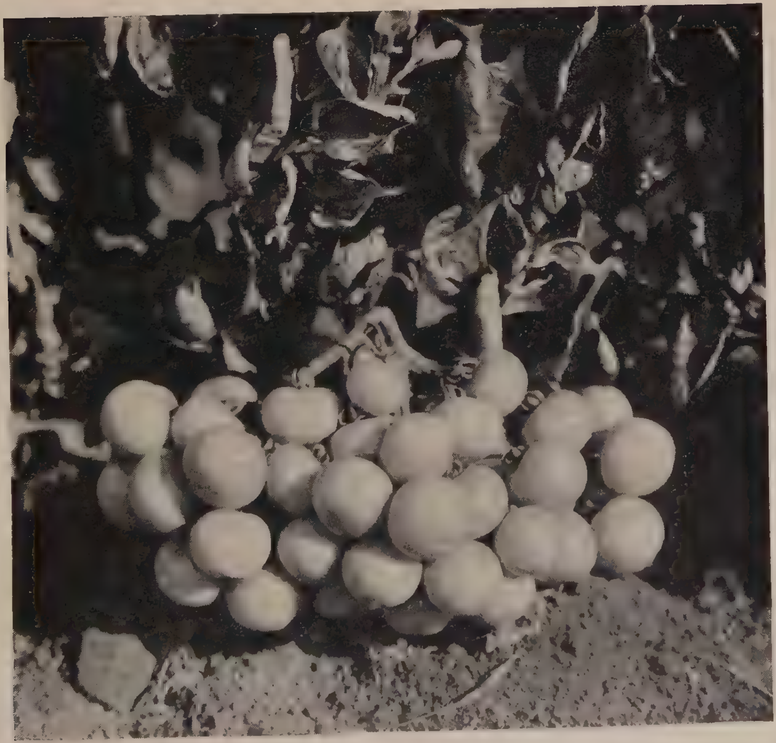

FIG. 2.

A neighbouring plant of which the bottom truss had been sprayed with BNP at 100 p.p.m. The truss carried 41 fruits of uniform size.

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A NOTE ON THE GROWTH-REGULATING ACTIVITY OF 2:6-DICHLOROPHENOXYACETIC ACID

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The fact that 2:4-dichlorophenoxyacetic acid (2,4-D) is an extremely potent growth-regulating substance prompted Swarbrick to investigate the activity of its isomeride, 2:6-dichlorophenoxyacetic acid (2,6-D). A sample, then believed to be the latter material, prepared by Dr. J. H. Mitchell, was found to possess activity approximately equal to that of 2,4-D. Thus each of these substances was shown by Swarbrick (1944, 1947) to set tomatoes at 10 p.p.m. and to set certain varieties of pear and apple at concentrations down to 30 p.p.m.

We have confirmed that the sample of 2,6-D used by Swarbrick possesses marked activity as a growth substance, being active in setting tomatoes in concentrations as low as 2·5 p.p.m. The same sample, sprayed at a concentration of 4 p.p.m., formed treatment DCA in the 1947 tomato setting trial described in the previous paper.

We have since discovered, however, that the material used was not, in fact, a pure sample of 2,6-D but consisted of a eutectic mixture of sharp melting point containing 42 per cent. of 2,4-D and 58 per cent. 2,6-D. Further, we have investigated a sample of pure 2,6-D and found it to be inactive in setting tomatoes and inactive in the Went pea test at concentrations up to 50 p.p.m.

It is clear, therefore, that the activity found by Swarbrick in the original sample must be largely attributed to the 2,4-D which was present in that material.

EXPERIMENTAL.

"2,6-D" used by Swarbrick. (I.) Phenol was chlorinated using the method of Chandelon (1883). One of the fractions obtained on distillation of the product had b.p. 217-219° C., and m.p. 63° C., and was considered to be 2:6-dichlorophenol. Condensation of this with chloracetic acid gave an acidic product obtained as white needles from water, m.p. 109·5-110·5° C., not affected by repeated crystallization.

Found Cl 32·07 per cent.; $C_8H_6O_3Cl_2$ requires 32·08 per cent.

Authentic 2,6-D. (II.) (Prepared by Mr. F. E. Smith.) The phenol, m.p. 59-60° C., was obtained by the method of Huston and Neley (1935). Condensation with chloracetic acid yielded 2:6-dichlorophenoxyacetic acid, white needles from water, m.p. 135-136° C.

Authentic 2,4-D. (III.) Prepared by condensing 2:4-dichlorophenol with chloracetic acid. White needles from water, m.p. 141° C.

Both II and III converted to their amides yielded pure products, m.p. 154-156° C. and 143-145° C. respectively. I, however, similarly treated, gave a mixture of amides, m.p. 120-139° C., from which 2:4-dichlorophenoxyacetamide was readily isolated by crystallization.

That I consisted of a mixture of II and III was confirmed by determining the melting points of a graded series of mixtures of II and III. The melting point curve revealed the presence of a simple eutectic at 110° C. corresponding to 42 per cent. 2,4-D and 58 per cent. 2,6-D. Addition of I to the eutectic mixture caused no alteration in the melting point.

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DATA FOR THE STUDY OF THE METABOLISM OF APPLES DURING GROWTH AND STORAGE*

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INTRODUCTION.

In this paper data are presented from a number of experiments on the metabolism of apples during growth and storage, which have been carried out at the Low Temperature Research Station, Cambridge, and at the Ditton Laboratory, Kent, during a period of twenty years by a team of workers of the permanent staff.

Many of the estimations have been replicated in order to obtain a high degree of accuracy but, owing to the great mass of figures involved, these are not shown. The standard deviations of the measurements have also been calculated.

These data will form the basis of more detailed papers which will follow in due course and which will deal with special aspects of the metabolism of the apple fruit.

The paper is divided into two parts. *Section I* contains data from experiments on the metabolism of apple fruits, designed by Dr. F. Kidd and Dr. C. West, the chemical analyses for which were carried out by Miss D. G. Griffiths and Miss N. A. Potter. *Section II* contains data on the nitrogen content and respiration of the apple fruit, obtained by Dr. A. C. Hulme.

SECTION I.

By D. G. Griffiths and N. A. Potter.

In 1928, when the first of these experiments was begun, the chief aim was the determination of carbohydrates, acidity and nitrogen and their correlation with respiration measurements but, as the work progressed, the scheme of analysis was widened to include other determinations and the substitution of new methods for those previously used.

In six of the eight experiments Bramley's Seedling apples were used, and the changes in composition during growth were studied by picking the fruit at different stages of maturity.

The gathering dates ranged from 19th June, when the average weight of the apples was 9·2 gm., to 24th October, when the average weight was 202·7 gm.

In the other two experiments Worcester Pearmain apples were used, the picking dates being 18th July and 4th September, when the weight of individual fruits averaged 25·6 and 70 gm. respectively. An outline of these experiments is shown in Table I.

Great care was exercised, both in the collection of the apples from the orchard and in the selection of the samples in order to reduce the sampling error to a minimum. Details of this procedure and also of the methods of storage and the preparation of samples for analysis are given under the appropriate headings.

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TABLE I.

No.	Variety.	Date of picking.	Size of fruit (gm.)	Number of apples in sample.	Temperature of storage ° C.	Conditions of storage.
I	Bramley's Seedling					
	(a)	28.9.28	168·5	60	−1°, 1°, 3°, 5°, 10°, 15°, 22·5°	air. air.
	(b)	24.10.28	202·7	60	5°	
II	Bramley's Seedling					
	(a)	31.8.31	112·6	30	10°	air.
	(b)	1.10.31	106·2	28	10°	air.
III	Worcester Pearmain	4.9.31	70	30	1°, 10°	air.
IV	Bramley's Seedling	23.6.33	30	44	3°, 10°	air.
V	Bramley's Seedling	10.7.34	30	40	1°, 3°, 10°, 15°, 20°	air, also (at 10° C. only) in 100% N, 100% O_2, and 20% O_2 + 10% CO_2 + 70% N_2.
VI	Worcester Pearmain					
	(a)	18.7.35	25·6	30	10°	air.
	(b)	29.7.35	37·2	30	10°	air.
	(c)	15.8.35	62·8	30	1°, 10°	air.
	(d)	2.9.35	69·8	30	10°	air.
VII	Bramley's Seedling	24.9.37	135·7	20	1°, 5°, 10°, 15°	air and in $\frac{1}{500}$ ethylene at 10° C.
VIII	Bramley's Seedling					
	(a)	19.6.39	9·2	100	10°	air.
	(b)	24.7.39	52·0	50	10°	air.
	(c)	14.8.39	72·1	30	10°	air.
	(d)	2.10.39	108·6	25	10°	air.

SELECTION OF SAMPLES FROM THE ORCHARD.

Two methods of gathering and sampling the fruit were adopted :

(*a*) In Experiment I all the apples from 15 trees of each of 4 rootstocks (Malling II, III, V and VII) were gathered, the fruits from each tree being kept separate. The apples were then brought to the laboratory as quickly as possible and the fruits from each tree were graded, one apple from each average-sized group from each tree being taken to form a sample of 60 fruits. Thus each sample was composed of an equal number of apples from each type and their weight was approximately equal. Imperfect fruits and extremes of size were rejected.

(*b*) In the other experiments, an average-sized apple was selected from the tree and all fruits judged visually to be approximately of that size were picked from a known number of trees and were then brought to the laboratory as quickly as possible. Owing to the variation in eye judgment, apples were found to differ somewhat in size so that grading was necessary ; samples were then made up of an equal number of apples from each grade and were of an approximately equal weight. In Experiment II the average weight of apples in the later picking is lower than that in the earlier picking. This is due to variation in visual selection, resulting in a greater number of smaller apples being included in the sample in the later picking, thus producing a lower average weight.

STORAGE OF SAMPLES.

Each sample was weighed, and a number of samples, known as the "initial" samples, were frozen to —20° C. immediately. The others were placed on wooden trays (one sample to a tray) in storage cabinets which stood in constant-temperature rooms and from which the apples were removed at pre-determined dates and weighed.

The top of the type of storage cabinet used in Experiments I-IV was a movable metal sheet, so that when the cabinet was full the top rested on the uppermost trays, and, when these were removed, it could be lowered to rest on the next layer of trays. In this way the air space round the apples was kept approximately the same and no artificial ventilation was necessary owing to the loosely fitting sides.

The trays were arranged in the cabinet in two tiers and were moved round, anti-clockwise, twice a week so that the effect of any variation of temperature was reduced to a minimum.

In Experiments V-VIII the storage cabinets were gas-tight metal boxes of 140 litres capacity, through which fresh air from outside the building could be drawn at a rate dependent on the weight of fruit present. The air was scrubbed free from CO_2 by soda-lime and was humidified in a calcium chloride tower. These cabinets held 12 trays, in 3 tiers of 4 trays each. The advantage of this form of cabinet is that the respiratory activity of the samples used for chemical analysis can be determined directly, instead of having to use a parallel sample, as was the case in the earlier experiments.

PREPARATION OF SAMPLES FOR ANALYSIS.

At the end of the storage periods the samples were weighed and the fruit treated according to one of the following methods.

(1) The apples were placed in airtight tins and taken immediately to a constant-temperature room held at —20° C. where the sample remained until required for analysis ; the fruit was then peeled and cored in the frozen state, only the pulp being used. This method was used in Experiments I, II, III and IV.

(2) In Experiments V and VI, the apples were cut in halves, and the pips removed. The samples were re-weighed and frozen in airtight tins at —20° C.

(3) The apples were cored in Experiment VII ; the cored apples were re-weighed before freezing in airtight tins at —20° C.

(4) In Experiment VIII, the apples were neither peeled nor cored, and were frozen whole in airtight tins at —20° C.

After this procedure, the apples were ground to a fine powder while still in the frozen state, by grating in a large electrically operated grating machine. The whole of the frozen powder from one sample was thoroughly mixed and then aliquots of 50 and 100 gm. were weighed out and placed in covered porcelain jars at —20° C. until required.

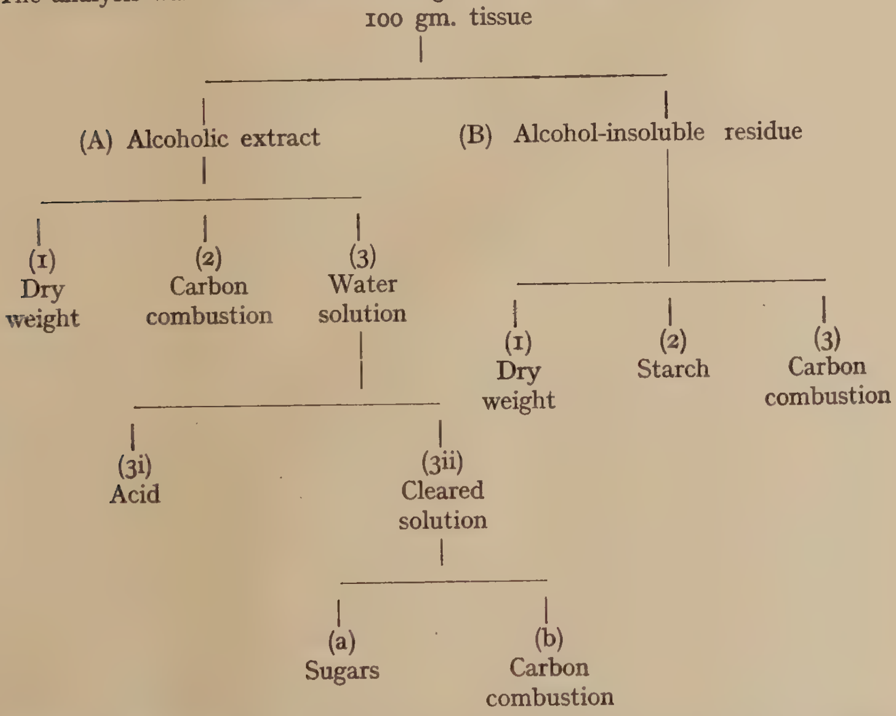
LOSS OF WEIGHT DURING STORAGE.

The apples which were stored showed a continuous loss of weight throughout their storage life. This loss was determined from the weights of the samples when they were removed from the store and a correction for this loss was made in all cases, so that the values are all finally referred back to unit original fresh weight and recorded in the tables as gm. per 100 gm. fresh weight. In cases where an apple had rotted completely it was removed from the sample but, when only small portions had rotted, these were cut out and the rest of the apple was used. The loss of weight was then calculated on the number of sound apples, allowance being made for the rotted fruit.

In Experiments I-V a single sample was used throughout the experiment for the determination of the loss of fresh weight ; it was weighed each time a sample was taken out of storage and was then replaced in the cabinet. This method had the disadvantage of introducing not only a sampling difference but also a tendency for a higher loss than that of the sample used for analysis, which was presumably due to the handling and frequent removals from storage for weighings. For this reason the actual samples used for analysis in Experiments VI-VIII were weighed at the time they were withdrawn from store and were re-weighed before being ground, since it was found that there was a further small loss of weight on freezing. The latter weight was used in the correction applied to bring values to original fresh weight.

METHODS OF ANALYSIS.

The analysis was carried out according to the following schema :


100 gm. tissue
(A) Alcoholic extract
(B) Alcohol-insoluble residue
(1) Dry weight
(2) Carbon combustion
(3) Water solution
(3i) Acid
(3ii) Cleared solution
(a) Sugars
(b) Carbon combustion
(1) Dry weight
(2) Starch
(3) Carbon combustion

ALCOHOLIC EXTRACTION.

100 gm. of frozen apple powder were used for extraction and were transferred in portions as quickly as possible to 200 ml. boiling 95 per cent. alcohol which was kept boiling for about 5 minutes after the final addition of powder and was then filtered through a large weighed fluted filter paper (No. 50 Whatman). The filter papers were

previously extracted with boiling alcohol, dried and weighed separately in glass-stoppered weighing bottles. The filter paper containing the apple tissue was then placed in a percolator fitted with a reflux condenser and extraction flask and extracted with 100 ml. hot 80 per cent. alcohol for 4 hours. In Experiment I, the alcoholic extract was kept neutral with NH_4OH during the transference of the apple to the alcohol, but as it was found that no hydrolysis of sucrose took place when neutralization was omitted and that the presence of NH_4OH interfered with the estimation of glucose and fructose, this procedure was abandoned.

(A) *Alcoholic extracts.* These were combined, and in Experiments I-V where alcoholic dry weights and carbon determinations were omitted, the alcohol was distilled off under reduced pressure at a temperature below 30° C. and the residue taken up in water and made up to a specified volume, usually 200 ml. In Experiments VI-VIII where carbon combustion and alcoholic dry weights were determined, the alcoholic extracts were combined, cooled and made up to 500 ml. or 1 litre with 80 per cent. alcohol. Then aliquots were taken for (1) and (2) and the remainder treated as described above for Experiments I-V.

(B) (i) *Alcohol-insoluble residue.* This was transferred in the filter paper to the weighing bottle and dried at 100° C. for 20 hours, when the weight was found to be constant.

(A) (3 ii) *Clarification of the aqueous solution.* An aliquot of the aqueous solution, when previously neutralized during extraction in Experiment I, was cleared with basic lead acetate, filtered, and the filtrate de-leaded with a saturated solution of potassium oxalate. In all other experiments an aliquot of the aqueous solution was neutralized with N.NaOH, cleared with basic lead acetate, filtered and the filtrate de-leaded with a saturated solution of sodium phosphate. In both cases the solution was made up to a suitable volume with water and filtered through a dry hardened filter paper. The filtrate was used for sugar determinations.

The inversion of sucrose.

In Experiments I, II, and III the sugar solution was inverted by boiling with 10 per cent. citric acid for 20 minutes. The solution was then cooled, neutralized with NaOH, and made up to a suitable volume for the determination of total reducing sugars. For Experiments IV-VIII, in which sugars were estimated by the micro-method, sucrose was hydrolysed according to Maskell's method, by holding the solution at 60° C. for 25 minutes in 0·5 N HCl, with subsequent neutralization for sugar determination.

(*a*) *Determination of sugar.*

Two methods were used for the determination of reducing sugars before and after the inversion of sucrose.

Method I. Lane and Eynon's method (1).

Method II. Maskell's modification of the Shaffer-Hartman micro-method (2). For the separate estimations of glucose and fructose, the former was estimated by the method of Hinton and Macara (3), whilst an adaptation of Kolthoff's method was used for the direct determination of fructose (4).

Method I was used in Experiments I, II and III for the determination of reducing sugars, in conjunction with Hinton and Macara's method for the direct estimation of glucose. Lane and Eynon's method is a volumetric one, based on the reduction of Fehling's solution, using methylene blue as an internal indicator. All the results obtained were calculated as invert sugar, using their tables. Glucose was estimated by hypoiodite according to the method of Hinton and Macara and, by combining this result, as described by Evans (5), with that obtained for reducing sugars, the amounts of glucose and fructose could be calculated.

It was found that the hypoiodite method gave a slightly high value for glucose owing to the presence of small quantities of substances other than glucose which were not removed by clarification and which were oxidized by hypoiodite. These substances were present in greater quantities in young apples than in mature ones. For this reason the direct estimation of fructose was adopted in Experiments IV to VIII, and at the same time a micro-sugar estimation was substituted for Method I as being more suitable for the estimation of the small quantities of reducing sugars found in immature fruits.

Method II: The Shaffer-Hartman micro-method which was used and modified by Dr. Maskell of the Botany School, Cambridge, is based on the reoxidation of cuprous oxide, produced by the action of reducing sugars on the copper reagent by a known amount of iodine, and the titration of the excess iodine with standard sodium thiosulphate solution.

The modified reagent consists of:

$$\left.\begin{array}{rl} 5{\cdot}0 & \text{gm. } CuSO_4\ 5H_2O \\ 10{\cdot}6 & \text{gm. } Na_2CO_3 \text{ (anhyd.)} \\ 16{\cdot}8 & \text{gm. } NaHCO_3 \\ 14{\cdot}1 & \text{gm. NaK Tartrate} \\ 18{\cdot}4 & \text{gm. K oxalate} \\ 0{\cdot}7 & \text{gm. } KIO_3 \end{array}\right\} \text{ in 1 litre.}$$

Alkaline solution for washing nitrogen:

$$\left.\begin{array}{rl} 10{\cdot}6 & \text{gm. } Na_2CO_3 \text{ (anhyd.)} \\ 16{\cdot}8 & NaHCO_3 \end{array}\right\} \text{ in 1 litre.}$$

5 ml. of this reagent were mixed in a boiling tube with 5 ml. of the sugar solution containing from 0·5 mg. to 1·25 mg. reducing sugars; nitrogen, which had previously passed through the alkaline solution, was bubbled through the mixture for 10 minutes. Bulb covers were placed on the tubes which were heated in a rapidly boiling water-bath for 15 minutes, then cooled for 5 minutes in running water, 2·5 ml. 2 per cent. KI solution and 2·5 ml. 2 $N.H_2SO_4$ were added, well mixed, allowed to stand for three minutes, and then titrated with N/75 Na thiosulphate solution in an atmosphere of N_2, using 1 per cent. starch solution as indicator. A blank titration was made, using 5 ml. of water in place of the sugar solution. The sugar present is calculated as glucose, using the following factor:

(ml. of 0·005 $N.Na_2S_2O_3$ used for blank—ml. of 0·005 $N.Na_2S_2O_3$ used for sugar solution) × 0·1125 = mg. of glucose.

For the direct estimation of fructose the method used was adapted from that described by Kolthoff (4). The glucose present was oxidized by alkaline hypoiodite, the excess iodine removed by the careful addition of sodium sulphite solution, and the fructose determined after neutralization by the micro-method already described. For convenience the result is calculated as glucose but can be subsequently converted to fructose by multiplying by the factor 1·03.

(3 i) *Determination of acidity.*

Determinations of acidity have been limited to measurements of titratable acidity, and as malic acid is the chief acid constituent of the apple, all calculations have been made in terms of this acid.

The acidity was determined by titrating an aliquot of the aqueous solution A (3) with 0·1 N.NaOH, using phenolphthalein as an indicator. It was found advisable to dilute the solution to at least twice its volume so that the end point could be seen more easily, the colouring matter of the apple tending to obscure it.

B (2) *Determination of starch.*

The determination of starch was carried out in all experiments by hydrolysis with taka-diastase and estimation of the reducing sugar produced. "Undiluted Taka-diastase" produced by Parke, Davis & Co. was used in every case.

In Experiments I-IV, the whole of the alcohol-insoluble residue from 100 gm. of apple was heated with 150 ml. of water in a boiling water-bath for half an hour, cooled, and according to the amount of starch present, from 0·1 to 0·3 gm. of taka-diastase in 10 ml. of water was added, together with 0·1 ml. 5 per cent. acetic acid and 2 ml. of toluol. The mixture was then incubated in an oven at 37° C. for 24 hours after which it was heated in a boiling water bath for 30 minutes to destroy the enzyme, filtered hot through a sintered glass funnel, the filtrate and washings cooled, and cleared as described in A (3ii).

The reducing sugar was then determined by the Shaffer Hartman micro-method (2), and related to the reducing value of a pure hydrolysed starch.

In Experiment V, when the micro-determination of sugars was adopted, the weight of alcohol-insoluble residue used was 0·5 to 1·0 gm., depending on the amount of starch present. The hydrolysis was similar to that described above but the estimation of reducing power was carried out by means of Maskell's modification of the Shaffer-Hartman micro-method and was then related to the reducing power of a pure hydrolysed apple starch.

At the end of the storage periods it was found that although there was no starch present which could be detected with iodine, a small value was always obtained in the starch determinations. This was called the "non-starch" fraction, and could be considerably reduced in value if the residue were shaken with potassium oxalate solution prior to the taka-diastase hydrolysis, as described by Widdowson (6). The weighed quantity (0·5-1·0 gm.) of insoluble residue was shaken therefore with 100-200 ml. of 1 per cent. solution of potassium oxalate for 5 hours, filtered with suction and washed free from oxalate with cold water, and then hydrolysed as described above. This treatment with potassium oxalate was used in Experiments VI, VII, and VIII, as it was found that the value after extraction agreed with that obtained by hydrolysis of the residue with β-amylase, which is specific for starch (7).

DETERMINATION OF DRY WEIGHT.

Two methods for the determination of the dry weight of the apple were used:

Method I. The method used in Experiments I-IV was to dry the frozen powder on weighed porous plates by quickly transferring a 50 gm. sample to the plate and spreading it in a thin even layer over the surface. The sample was then dried in a large air oven at 50° C. for 48 hours, cooled and weighed. The same number of samples was dried each time, so that the drying conditions remained as constant as possible. This method was eventually given up as it was found impossible to procure uniform porous plates when new supplies were required.

Method II. In Experiments V to VIII, the dry weight was determined as the sum of the dry weights of the alcohol-insoluble residue B (i) and the alcohol-soluble residue A (i). The dry weight of the insoluble residue was obtained by the procedure described in the method for extraction. For the alcohol-soluble dry weight 10 ml. aliquots were taken from the measured alcoholic extract, placed in weighed glass dishes provided with lids, and dried in an air oven at 50° C. for 48 hours, after which they were cooled and weighed.

The sum of these results showed good agreement with those obtained by the direct drying of the pulp.

DETERMINATION OF CARBON.

In order to follow the change in the total carbon content of the apple during storage and to compare it with the estimation of carbon loss by respiration, the total carbon present was determined in Experiment VII and in parts of Experiments VI and VIII.

The method used was the wet combustion method employed by Raistrick (8) with the modification of apparatus introduced by Maskell. Owing to the difficulty of weighing accurately small quantities of frozen apple powder at —20°, the carbon determination had to be made in two parts: (1) in the alcohol-soluble extract (A2), and (2) in the alcohol-insoluble residue (B3). The sum of (1) and (2) represents the total carbon content of the apple. Carbon was also determined in the cleared solution (A3ii), allowance being made for the carbon present in the volume of basic lead acetate added for clearing.

A2. *Carbon content of alcoholic extract.*

The method was standardized to estimate approximately 20 mg. of carbon. 5 to 10 ml. of the original total alcoholic extract were used according to the sugar content, placed in a specially adapted Klaisen flask and the alcohol evaporated under reduced pressure in a water bath at a temperature not exceeding 30° C. Any remaining traces of alcohol were removed by the passage of a stream of air. 5 ml. of CO_2-free distilled water were added to bring the residue into solution and so facilitate oxidation. For the wet combustion, 5 ml. of 100 per cent. chromic acid (100 gm. chromic anhydride in 100 ml. solution) and 50 ml. of concentrated H_2SO_4 were added and heated in a stream of CO_2-free air. The products of combustion were passed over (1) finely divided CuO, (2) lead chromate and (3) a copper gauze spiral, all contained in a silica tube heated to dull redness in a combustion furnace. The CO_2 produced was drawn through a sintered glass spoon into a solution of 0·25 N baryta contained in a gas jar. When the oxidation was complete, the gas jar was removed and the contents titrated in the jar with 0·25 N HCl in a closed circuit, using phenolphthalein and then methyl orange as indicators. A blank estimation was run on the reagents. The results were expressed as grams of carbon per 100 gm. of fresh weight.

B (3). *Carbon content of the alcohol-insoluble residue.*

For the estimation of carbon in the alcohol-insoluble residue, approximately 0·05 gm. of the finely divided dried residue was weighed and carefully transferred to the combustion flask. 5 ml. of CO_2-free distilled water were added, and the oxidation carried out as described in A2.

For the determination of carbon in the cleared solution A(b), 5 to 10 ml. of the cleared solution were used and the same procedure was followed.

DETERMINATION OF NITROGEN.

Nitrogen determinations were made in only a few cases. In Experiment I, total nitrogen was estimated in every sample, but in Experiments II, III and V it was only determined in the initial samples. In addition, soluble nitrogen was estimated in a few storage samples in Experiment V.

Kjeldahl's method was used, but certain precautions were necessary in dealing with frozen apple tissue. 50 gm. of the frozen apple powder were carefully transferred to a 650 ml. pyrex Kjeldahl flask in the —20° C. room, and 30 ml. of concentrated H_2SO_4 added immediately in the laboratory. At first the mixture was heated carefully because of frothing. When this had ceased, 10 gm. of K_2SO_4 and 1 gm. of $CuSO_4$ and another 30 ml. of concentrated H_2SO_4 were added and the mixture heated until the liquid was almost transparent; then 5 ml. of H_2O_2 (100 vol.) were added and the heating continued until the solution was quite clear. Nitrogen was then estimated as NH_3 by steam distillation in the usual way.

Alcohol-soluble nitrogen was determined in some samples of Experiment V by extracting 100 gm. of frozen apple powder in a Soxhlet thimble with 85 per cent. alcohol at 50° C. in a vacuum extraction apparatus for 4½ hours. The extract was then evaporated on the water bath, and digested with concentrated H_2SO_4 and the same procedure followed as that described for total nitrogen.

METHODS FOR THE DETERMINATION OF THE CO_2 OF RESPIRATION.

Ideally the CO_2-production of each sample used for chemical analysis should be measured. In practice, the labour and equipment for doing this would be too great, so that the following methods have been used.

Method I. A special sample was used for the determination of CO_2-production in Experiment I and, as an additional method, in Experiment V (a) and (b). From the 60 apples comprising the sample, twelve were selected and the CO_2-output of each individual apple was observed throughout the storage period. This is termed the "continuous" method.

The respiration chamber used for single apples consisted of two flanged glass hemispheres of 300-500 ml. capacity, with tubulatures in the middle of their domes. CO_2-free air, which was conditioned to 80 per cent. humidity, was drawn through the chambers at a standard rate of 2 to 3 litres per hour; the CO_2 produced was absorbed by baryta in Pettenkofer tubes, which were changed at suitable intervals, and the baryta titrated with HCl, using phenolphthalein as indicator. The disadvantages of this procedure are that the apples remaining in the respiration containers throughout their storage period are under different conditions from those stored for chemical analysis, firstly, as regards ventilation and water loss, the average water loss of the chemical samples being 2-5 times greater than that of the respiration sample, and secondly, as regards the possible effect of one apple upon another in the case of the chemical samples stored in the cabinets, but not in the case of the apples used for the measurement of CO_2-production. That such effects do in fact occur has been shown elsewhere (9).

The advantages of the method are that it enables results to be submitted to statistical analysis and that when any one individual apple becomes affected by rot, which considerably increases the rate of CO_2-production, the respiration of the sound part of the sample can be distinguished from that of the rotting fruit. As stated earlier in the text, rotted parts of apples in the chemical samples were removed before freezing prior to analysis, since it has been shown that the excess CO_2-production accompanying rotting is attributable to the contact between the advancing rot and the sound tissue (10).

Method II. In this method, used in Experiments II, III, IV, V and VI, the whole sample allotted for the measurement of CO_2 was stored with the chemical samples and was divided into a number of aliquots. The expected storage life was then divided into a number of intervals corresponding to the number of sub-samples, and at each successive interval, a different sub-sample was used for the measurement of CO_2-production. The respiration of apples of the sub-sample was measured individually as described above. This procedure, which is termed the " successive group " method, was followed in order to retain the advantages and avoid the disadvantages of Method I.

The main disadvantage of Method II arises from the extra handling involved in removing the sub-samples, particularly if the fruits are beginning to wilt, when even the most gentle handling increases both CO_2-production and water loss.

Experiment II (b) illustrates the difficulty encountered due to the effect of one apple upon another when stored together in bulk. The rate of CO_2-production of an aliquot sample of 6 apples, each in its own container, which was brought under observation at the outset, fell for about 10 days before the climacteric rise began. When about halfway up the climacteric rise this sub-sample of 6 apples was replaced by the second sub-sample, which in the meantime had been in the chamber with the main bulk of chemical samples. The rate of CO_2-production of this second sub-sample indicated that the apples had already completed their climacteric rise, the probability being that their climacteric had started immediately after the beginning of their storage in bulk. The results for CO_2-production in this case are denoted by * in Experiment IIb.

In Experiments Va and b, the respiration was determined both by the " continuous " method and by the " successive group " method. The loss of water sustained by the apples for which the latter method was used was three times as great as that of the apples for which the " continuous " method was used ; in fact it corresponded more closely to the loss of water of the chemical samples.

Method III. The whole respiration sample or a large aliquot of the sample was stored in a desiccator and the CO_2-production measured by the method described by Hulme (11). This necessitated the use of NaOH instead of baryta for absorption of CO_2, and a more rapid flow of air through the apparatus in order to prevent an accumulation of CO_2 in the desiccator. The disadvantages of this method are that it is inadequate for a very large number of apples and that there is no means of distinguishing between the CO_2-production of the sound fruits and that of any rotting fruits in the sample. This method was used throughout Experiment VII.

Method IV. In this method the chemical samples were stored in a hermetically sealed chamber ventilated at a rate sufficient to keep the CO_2 in the atmosphere at a low level. The total CO_2-production of all the chemical samples was measured by the " split current " procedure, in which capillary tubes were used as differential resistances, so that the current of air leaving the storage chamber could be divided into a main large fraction and a second small one, the CO_2 in the latter only being measured by absorption by baryta in Pettenkofer tubes. For this purpose, the apparatus was arranged so that the rate of flow of the small fraction was approximately 2-3 litres an hour, which provides the conditions as regards accumulation of CO_2 similar to those obtaining in single containers of 3-500 ml. capacity.

This method was used for Experiment VII at 10° C. storage only, and in Experiment VIII, and probably gives the most satisfactory results, provided no rotting has occurred.

DETERMINATION OF VOLATILES.

The volatile substances, other than CO_2 and water vapour, produced by the apples, were determined by passing CO_2-free air over the sample of fruits, then through a soda-lime tube to absorb the CO_2 of respiration, and finally through a silica tube containing copper oxide heated in a furnace to dull red heat. The resultant CO_2 was absorbed in baryta in a Pettenkofer tube, and estimated by titration with HCl. The results were expressed as mg. of CO_2 per 10 kg. per hour.

CALCULATIONS OF STANDARD DEVIATIONS.

(1) *Chemical constituents.* The method used is described in Section II.

(2) *Respiration.* The standard deviation for methods (a) and (b) is shown in the tables. In Experiment I, curves were drawn to the average reading for each day for all apples together, and from these the total amount of CO_2 produced up to certain periods was obtained. The standard deviation of this estimate has been calculated from the standard deviation of the bounding ordinates.

In Experiments II to IV, the standard deviation for the CO_2-production of individual apples in the groups was calculated and expressed as a percentage. The mean of these percentages was determined and the standard deviation of this mean then estimated for the whole storage period.

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EXPERIMENT I.

Bramley's Seedling apples stored at seven temperatures.

Source: East Malling, Plot 10. 15 trees from each stock types 2, 3, 5, 7.

Gathering date 28.9.28. Number of apples per sample 60. Average weight 168·5 gms.

(a) *1st pick.*

Days in store	Relative fresh weight. gm.	Gm. per 100 gm. fresh weight.							Breakdown.	Rots.	Respiration.		Standard error as percentage.
		Glucose.	Fructose.	Sucrose.	Starch.	Titratable acidity as malic.	Dry weight.	Nitrogen.			Total CO_2 gm. per 100 gm.	Rate CO_2 ccs. per 10 K.H.	
Storage temperature −1°C.													
0	100·0	1·11	5·74	3·51	0·60	1·27	15·14	0·035	—	—	—	15	—
13	99·5	1·18	5·64	3·74	0·17	1·35	15·73	0·042	—	—	0·09	14	3·8
27	99·0	1·31	5·95	3·64	0·11	1·32	15·55	0·036	—	—	0·19	14	3·9
41	98·6	1·45	6·03	3·48	0·06	1·29	16·27	0·039	—	—	0·29	15	4·0
76	97·7	1·68	6·29	3·11	—	1·28	15·76	0·043	4%	—	0·53	17	5·0
111	96·9	1·77	6·10	2·71	—	1·18	15·63	0·041	37%	—	0·85	20	5·3
132	96·2	1·75	5·95	2·52	—	1·08	14·45	0·037	60%	—	1·06	21	5·7
153	95·5	1·77	5·96	2·43	—	1·00	14·94	0·037	85%	—	1·28	22	5·9
174	94·9	1·73	5·74	2·20	—	0·83	13·97	0·036	95%	—	1·50	22	5·9
Storage temperature +1°C.													
0	100·0	1·06	5·85	3·61	0·60	1·28	15·22	0·036	—	—	—	20	—
13	99·2	1·29	6·04	3·72	0·17	1·33	15·86	0·038	—	—	0·12	19	1·7
27	98·7	1·43	5·86	3·54	0·06	1·26	15·63	0·039	—	—	0·24	20	1·8
41	98·3	1·53	6·00	3·41	0·05	1·31	15·84	0·040	—	—	0·38	22	1·8
76	97·5	1·78	6·21	2·90	—	1·24	16·03	0·039	—	—	0·76	24	1·8
111	96·6	1·88	6·19	2·38	—	1·10	14·68	0·035	—	1	1·17	26	1·7
146	95·1	1·99	6·12	1·94	—	1·05	14·87	0·036	8%	—	1·62	28	—
186	93·8	2·02	6·05	1·63	—	0·88	13·80	0·034	20%	1	2·18	32	—
213	92·8	1·97	5·81	1·38	—	0·78	13·05	0·036	52%	—	2·58	30	—
Storage temperature +3°C.													
0	100·0	1·11	5·77	3·65	0·65	1·30	15·71	0·038	—	—	—	23	—
13	99·3	1·17	5·83	3·78	0·14	1·31	15·52	0·038	—	—	0·14	23	2·9
27	98·7	1·43	5·81	3·58	0·04	1·34	15·76	0·039	—	—	0·30	26	3·2
41	98·2	1·56	5·97	3·16	0·04	1·26	15·31	0·038	—	—	0·48	28	3·0
72	97·2	1·67	6·67	2·88	—	1·18	15·62	0·037	—	—	0·91	31	2·7
101	95·9	1·89	6·15	2·22	—	1·12	14·92	0·037	—	—	1·35	32	2·2
142	93·8	2·01	6·22	1·79	—	1·00	14·34	0·033	—	1	1·97	32	2·1
178	92·1	2·09	6·14	1·45	—	0·88	13·93	0·033	10%	6	2·51	30	—
247	84·9	2·05	5·73	1·03	—	0·75	12·17	0·032	20%	11	3·43	26	—
Storage temperature +5°C													
0	100·0	1·10	5·64	3·62	0·64	1·32	15·57	0·034	—	—	—	30	—
13	99·0	1·38	6·04	3·69	0·10	1·31	15·92	0·038	—	—	0·18	29	1·6
27	97·9	1·58	6·22	3·35	0·01	1·26	15·07	0·037	—	—	0·38	34	1·6
41	96·9	1·67	6·11	3·06	—	1·22	14·96	0·037	—	—	0·62	38	1·5
72	94·8	1·79	6·19	2·39	—	1·13	15·33	0·035	—	—	1·18	39	1·5
101	92·8	1·94	6·23	1·96	—	1·02	14·92	0·034	—	—	1·71	37	—
126	91·3	2·01	6·01	1·61	—	0·95	13·79	0·037	2%	3	2·14	35	—
161	89·3	2·09	6·20	1·22	—	0·87	13·36	0·035	2%	14	2·68	32	—
191	87·3	2·13	5·85	0·85	—	0·75	12·53	0·034	—	18	—	—	—
Storage temperature +10°C.													
0	100·0	1·13	6·13	3·51	0·60	1·39	15·74	0·039	—	—	—	48	—
11	99·0	1·36	5·82	3·65	0·07	1·31	15·87	0·039	—	—	0·23	50	4·7
21	98·4	1·62	6·32	3·14	—	1·27	15·33	0·037	—	—	0·52	66	4·9
31	97·5	1·65	6·21	2·95	—	1·22	15·34	0·038	—	1	0·83	62	5·0
51	94·9	1·96	6·60	2·27	—	1·14	15·07	0·036	—	1	1·39	55	4·5
76	92·4	2·06	6·56	1·57	—	0·98	14·78	0·037	—	2	1·98	45	—
107	90·6	2·23	6·35	1·16	—	0·91	13·71	0·036	—	18	2·60	39	—
116	89·6	2·26	6·13	0·86	—	0·82	13·52	0·036	—	22 (10 removed)	2·76	38	—
Storage temperature 15°C.													
0	100·0	1·18	5·56	3·56	0·58	1·32	15·69	0·037	—	—	—	73	—
6	99·3	1·36	5·94	3·50	0·08	1·32	15·41	0·038	—	—	0·18	65	4·2
14	98·3	1·56	6·12	3·22	—	1·26	15·52	0·037	—	—	0·51	101	3·9
23	97·3	1·74	6·02	2·79	—	1·17	14·96	0·037	—	1	0·94	94	3·3
32	96·3	1·97	6·36	2·13	—	1·05	15·05	0·036	—	—	1·32	83	3·1
46	94·3	2·04	6·39	1·61	—	1·00	14·62	0·037	—	—	1·82	70	3·0
60	92·8	2·18	6·39	1·14	—	0·92	14·52	0·037	2%	9	2·25	59	3·0
73	91·3	2·30	6·68	0·92	—	0·87	14·03	0·039	—	9	2·60	54	—
79	89·4	2·38	6·32	0·71	—	0·76	13·79	0·038	—	13	2·75	53	—
Storage temperature 22·5°C.													
0	100·0	1·23	5·75	3·57	0·64	1·32	15·47	0·037	—	—	—	114	—
5	99·1	1·66	6·23	3·15	0·11	1·27	15·63	0·039	—	—	0·23	95	4·9
12	97·9	1·36	6·40	2·25	—	1·11	14·86	0·039	—	—	0·73	172	4·8
18	96·6	2·09	6·49	1·78	—	1·00	14·41	0·039	—	1	1·20	154	3·9
24	95·4	2·15	6·58	1·32	—	0·86	14·40	0·037	—	3	1·61	130	3·4
30	94·4	2·24	6·56	1·06	—	0·76	14·27	0·039	—	—	1·96	113	3·2
40	92·9	2·29	6·55	0·66	—	0·59	13·61	0·036	—	2	2·45	94	3·1
51	90·9	2·28	6·61	0·47	—	0·48	13·38	0·037	—	—	2·89	79	—
72	88·2	2·20	6·42	0·26	—	0·34	13·53	0·037	—	16	3·63	72	—
Standard deviation of 7 initial samples expressed as percentage of each constituent ..		5·0	3·1	1·5	4·4	3·0	1·5	4·8	—	—	—	—	—
Standard deviation of duplicate estimations ..		2·2	0·36	1·1	—	0·7	0·3	1·3	—	—	—	—	—

(b) *2nd pick.*

Gathering date 24.10.28. Number of apples per sample 60. Average weight 202·7 gms.

Storage Temperature 5°C.													
0	100·0	1·41	5·87	4·07	—	1·23	15·82	—	—	—	—	33	—
10	99·3	1·50	6·06	3·84	—	1·19	15·68	—	—	—	0·16	36	—
41	98·2	1·64	6·11	3·25	—	1·17	16·47	—	—	2	0·70	37	—
60	97·3	1·85	6·28	2·83	—	1·09	15·73	—	—	2	1·04	37	—
91	96·3	1·98	6·31	2·16	—	0·95	14·76	—	2%	4	1·59	34	—
120	95·2	2·22	6·41	1·73	—	0·90	14·40	—	—	27	2·15	31	—
148	94·6	2·16	6·37	1·43	—	0·82	13·60	—	—	53	2·80	29	—

Experiment II.

Two gatherings of Bramley's Seedling apples stored at 10° C. in air.
Source: Shippea Hill, Ely.

(a) *1st pick.*

Gathering date 31.8.31. Number of apples per sample 30. Average weight 112·6 gms.

Days in store.	Relative fresh weight gm.	Gm. per 100 gm. fresh weight.						Rots.	Respiration.		Standard error as percentage
		Glucose.	Fructose.	Sucrose.	Starch.	Titratable acidity as malic.	Alcohol insoluble residue.		Total CO_2 gm. per 100 gm.	Rate CO_2 ccs. per 10 K.H.	
0	100·0	1·15	3·44	1·76	1·50	1·65	3·48	—	—	—	—
0	100·0	1·14	3·23	1·68	1·20	1·66	3·35	—	—	—	—
0	100·0	1·25	3·12	1·60	1·27	1·67	3·38	—	—	—	—
Storage temperature 10°C.											
3	99·63	1·09	3·32	1·65	0·90	1·69	3·14	—	0·08	52	3·4
7	99·30	1·26	3·43	1·89	0·68	1·69	2·77	—	0·17	47	
11	99·01	1·41	3·38	1·81	0·28	1·67	2·31	—	0·26	44	
15	98·79	1·43	3·62	1·86	0·16	1·66	2·21	—	0·34	43	
21	98·48	1·53	3·69	1·67	0·07	1·68	2·05	—	0·46	44	
29	98·06	1·68	3·94	1·57	0·09	1·54	2·03	—	0·64	48	
37	97·63	1·72	3·81	1·10	0·08	1·47	1·96	—	0·83	53	
49	97·17	1·74	4·02	1·09	—	1·45	2·00	—	1·14	58	
61	96·22	1·79	3·97	0·87	—	1·42	2·02	—	1·47	59	
73	95·37	1·74	4·11	0·68	—	1·33	1·94	—	1·81	57	
88	94·46	1·80	4·03	0·59	0·08	1·25	1·92	—	2·20	53	
105	93·28	1·72	4·16	0·46	—	1·20	1·94	2 rots removed.	2·60	51	
125	91·78	1·68	4·15	0·34	—	1·05	1·89	2 small rots cut out.	3·08	49	
147	90·30	1·53	3·66	0·34	0·07	0·97	1·82	1 rot removed.	3·59	48	
178	88·12	1·42	3·81	0·23	0·06	0·83	1·84	1 rot removed.	4·28	47	
Changed from 10°C. *to* 1°C. *after* 21 *days at* 10°C.											
21 +											
5	98·26	1·77	3·50	1·43	—	1·63	1·99	3 small rots cut out.	0·54	22	—
10	98·12	1·72	3·95	1·56	—	1·68	2·08	1 rot removed.	0·58	18	—
15	97·97	1·71	3·72	1·50	—	1·60	2·05	—	0·62	19	—
22	97·79	1·76	3·93	1·55	—	1·60	2·01	—	0·69	21	—
29	97·50	1·67	4·03	1·43	—	1·59	2·06	—	0·76	22	—
36	97·40	1·68	3·87	1·25	—	1·55	2·01	—	0·83	24	—
Standard deviation of 3 initial samples expressed as percentage of each constituent		5·1	5·0	4·7	11·9	0·6	2·0	—	—	—	—
Standard deviation of duplicate samples . .		3·0	1·3	1·1	—	0·3	—	—	—	—	—

(b) *2nd pick.*

Gathering date 1.10.31. Number of apples per sample 28. Average weight 106·2 gms.

0	100·0	1·40	4·36	2·56	0·45	1·48	2·38				
0	100·0	1·22	4·30	2·65	0·50	1·46	2·49				
0	100·0	1·41	4·28	2·60	0·37	1·50	2·34				
Storage temperature 10°C.											
4	99·63	1·45	4·25	2·60	0·29	1·46	2·24	—	*0·10	52	5·8
8	99·20	1·60	4·48	2·52	0·12	1·41	2·01	—	*0·20	55	
12	98·89	1·76	4·33	2·06	0·09	1·43	1·92	—	*0·31	58	
16	98·57	1·64	4·43	2·07	0·08	1·39	1·86	—	*0·42	59	
22	98·10	1·70	4·53	1·96	0·09	1·37	1·90	—	*0·58	60	
30	97·50	1·75	4·53	1·73	0·08	1·29	1·86	—	0·81	60	
39	96·79	1·89	4·60	1·38	—	1·24	1·86	—	1·06	59	
50	95·98	1·79	4·62	1·30	—	1·21	1·88	—	1·36	56	
62	94·56	1·84	4·78	1·10	—	1·12	1·85	—	1·67	54	
74	93·58	1·84	4·54	0·92	—	1·09	1·78	1 small rot cut out.	1·97	52	
89	92·15	1·84	4·52	0·68	0·08	1·02	1·78	1 small rot cut out.	2·33	50	
105	90·76	1·89	4·73	0·59	—	0·96	1·75	1 rot removed.	2·70	48	
126	89·11	1·79	4·50	0·47	—	0·87	1·72	1 rot removed.	3·17	45	
147	87·53	1·80	4·31	0·36	0·07	0·79	1·69	1 rot removed and 5 small rots cut out.	3·61	44	
168	80·18	1·50	4·02	0·28	0·06	0·66	1·56	2 rots removed and 7 small rots cut out.	4·04	43	
Changed from 10°C. *to* 1°C. *after* 31 *days at* 10°C.											
31 +											
5	97·18	1·75	4·67	1·79	—	1·23	1·88	—	0·89	21	—
10	97·02	1·90	4·58	1·39	—	1·29	1·85	—	0·94	22	—
15	96·89	1·73	4·72	1·60	—	1·31	1·89	—	0·99	22	—
22	96·70	1·78	4·73	1·68	—	1·27	1·93	—	1·06	22	—
29	96·54	1·80	4·74	1·50	—	1·27	1·92	—	1·13	23	—
36	96·32	1·85	4·70	1·28	—	1·22	1·90	—	1·21	24	—
Standard deviation of 3 initial samples expressed as percentage of each constituent		7·9	0·9	1·7	14·9	1·3	3·2	—	—	—	—

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* See note on page 275.

EXPERIMENT III.

Worcester Pearmain apples stored at 10° C. and 1° C.

Source: Cottenham, Cambridge.

Gathering date 3.9.31. Number of apples per sample 30. Average weight 70 gms.

Days in store.	Relative fresh weight gm.	Gm. per 100 gm. fresh weight.						Rots.	Respiration.		Standard error as percentage.
		Glucose.	Fructose.	Sucrose.	Starch.	Titratable acidity as malic.	Alcohol insoluble residue.		Total CO_2 gm. per 100 gm.	Rate CO_2 ccs. per 10 K.H.	
0	100·0	1·56	5·83	1·92	0·58	0·56	2·66	—	—	—	—
0	100·0	1·55	5·90	1·93	0·49	0·56	2·61	—	—	—	—
0	100·0	1·49	5·97	1·85	0·51	0·56	2·69	—	—	—	—
0	100·0	1·58	5·85	1·81	0·62	0·56	2·75	—	—	—	—
Storage temperature 1°C.											
6	99·13	1·47	5·73	1·75	0·41	0·52	2·38	—	[illegible]	[illegible]	[illegible]
11	98·78	1·59	5·88	1·81	0·35	0·54	2·36	—	[illegible]	[illegible]	
21	98·09	1·72	6·15	1·37	0·22	0·52	2·23	—	[illegible]	[illegible]	
33	97·39	1·78	6·01	1·24	0·17	0·48	2·12	—	[illegible]	[illegible]	
48	96·69	1·76	6·35	1·18	0·16	0·47	2·09	—	[illegible]	[illegible]	
63	96·11	1·72	6·44	0·94	0·12	0·43	2·08	—	[illegible]	[illegible]	
84	95·49	1·66	6·80	0·79	0·15	0·38	2·07	—	[illegible]	[illegible]	
105	94·60	1·50	6·99	0·50	—	0·36	2·10	—	[illegible]	[illegible]	
126	94·06	1·38	6·64	0·35	—	0·31	2·03	1 small rot cut out.	[illegible]	[illegible]	
147	92·89	1·42	7·03	0·38	—	0·30	2·08	1 small rot cut out.	[illegible]	[illegible]	
168	91·88	1·24	6·91	0·33	—	0·21	1·97	—	[illegible]	[illegible]	
190	90·02	1·10	6·83	0·27	0·14	0·19	1·96	1 rot removed and 2 small rots cut out.	[illegible]	[illegible]	
Samples changed from 1°C. *to* 10°C. *after* 104 *days at* 1°C.											
104 +											
7	93·71	1·34	6·69	0·50	—	0·34	2·00	—	[illegible]	[illegible]	—
15	92·20	1·21	6·66	0·52	—	0·32	2·02	—	[illegible]	[illegible]	—
23	90·70	1·21	6·61	0·45	—	0·29	1·93	—	0·84	[illegible]	—
33	88·86	1·16	6·23	0·30	—	0·28	1·89	1 rot removed.	[illegible]	[illegible]	—
46	86·24	0·87	6·52	0·27	—	0·24	1·81	3 small rots cut out.	[illegible]	[illegible]	—
61	83·60	0·83	6·47	0·22	—	0·23	1·81	2 small rots cut out.	[illegible]	[illegible]	—
Storage temperature 10°C.											
3	99·14	1·66	5·66	1·63	0·40	0·55	2·41	—	[illegible]	[illegible]	[illegible]
8	98·04	1·73	6·14	1·65	0·22	0·54	2·20	—	[illegible]	[illegible]	
13	97·10	1·51	6·21	1·72	0·18	0·50	2·10	—	[illegible]	[illegible]	
18	96·52	1·74	6·29	1·32	0·20	0·52	2·12	—	0·70	[illegible]	
28	94·72	1·60	6·20	1·08	0·18	0·45	1·99	2 rots removed.	[illegible]	[illegible]	
40	93·89	1·42	6·31	0·80	0·15	0·43	1·97	—	[illegible]	75	
52	92·79	1·36	6·31	0·61	0·14	0·38	1·90	—	[illegible]	68	
64	91·73	1·34	6·47	0·41	0·15	0·36	1·91	—	[illegible]	61	
76	90·73	1·28	6·04	0·34	—	0·31	1·91	—	[illegible]	55	
88	89·50	1·20	6·13	0·28	—	0·30	1·89	—	[illegible]	52	
101	88·03	1·14	5·82	0·29	—	0·26	1·84	2 rots removed and 2 small rots cut out.	3·25	50	
113	86·36	1·12	5·79	0·21	0·16	0·25	1·89	1 rot removed and 9 small rots cut out.	[illegible]	[illegible]	
Samples changed from 10°C. *to* 1°C. *after* 8 *days at* 10°C.											
8 +											
5	97·62	1·77	5·88	1·47	0·17	0·54	2·15	—	[illegible]	[illegible]	—
11	97·26	1·74	6·04	1·31	0·17	0·50	2·09	1 rot removed.	[illegible]	[illegible]	—
21	96·66	1·66	6·21	1·27	0·17	0·50	2·12	—	[illegible]	[illegible]	—
33	96·13	1·75	6·47	1·18	—	0·47	2·04	—	[illegible]	[illegible]	—
47	95·42	1·64	6·35	0·89	—	0·43	2·02	—	[illegible]	[illegible]	—
61	94·90	1·53	6·60	0·67	—	0·41	2·00	—	[illegible]	[illegible]	—
82	94·23	1·45	6·89	0·56	—	0·36	2·00	5 small rots cut out.	[illegible]	24	—
103	93·30	1·38	6·82	0·42	—	0·32	1·99	—	[illegible]	23	—
125	92·53	1·39	6·52	0·31	—	0·27	2·02	—	[illegible]	22	—
Samples changed from 10°C. *to* 1°C. *after* 89 *days at* 10°C.											
89 +											
7	89·10	1·30	6·08	0·31	—	0·28	1·91	—	[illegible]	[illegible]	—
14	88·76	1·21	5·86	0·25	—	0·28	1·85	1 rot removed and 6 small rots cut out.	[illegible]	[illegible]	—
21	88·51	1·19	5·84	0·25	—	0·28	1·87	2 rots removed and 2 small rots cut out.	[illegible]	15	—
35	88·02	1·14	5·74	0·20	—	0·26	1·80	2 rots removed and 3 small rots cut out.	[illegible]	[illegible]	—
49	87·51	1·10	5·74	0·19	—	0·27	1·85	1 rot removed and 2 small rots cut out.	[illegible]	[illegible]	—
63	87·30	1·09	5·61	0·18	—	0·26	1·85	2 rots removed 5 small rots cut out.	[illegible]	[illegible]	—
Standard deviation of 4 initial samples expressed as percentage of each constituent		2·5	1·0	3·0	10·9	0·0	2·1	—	—	—	—

Experiment IV.

Bramley's Seedling apples stored at 3° C. and 10° C.

Source : Farningham, Kent.

Gathering date 23.6.33. Number of apples per sample 44. Average weight 30 gms.

Days in store.	Relative fresh weight gm.	Gm. per 100 gm. fresh weight.							Rots.	Respiration.		Standard error as percentage.
		Glucose.	Fructose.	Sucrose.	Starch.	Titratable acidity as malic.	Alcohol insoluble residue.	Dry weight.		Total CO_2 gm. per 100 gm.	Rate CO_2 ccs. per 10 K.H.	
0	100·00	0·66	1·82	0·75	0·93	2·47	4·14	12·92	—	—	—	—
in 3°	99·63	0·61	1·72	0·81	0·88	2·51	4·05	12·85	—	—	—	—
overnight	99·63	0·64	1·80	0·78	0·86	2·51	4·08	12·93	—	—	—	—
,,	99·63	0·63	1·77	0·74	0·90	2·47	4·03	12·65	—	—	—	—
Storage temperature 3°C.												
8	96·98	0·91	1·91	1·02	0·25	2·49	3·21	12·75	—	0·23	47	3·6
15	94·82	0·91	2·10	0·92	0·13	2·50	3·02	12·46	—	0·38	46	
24	92·03	0·96	2·06	0·69	0·12	2·41	2·78	11·81	—	0·60	59	
34	89·27	0·95	2·09	0·56	0·11	2·30	2·68	11·60	—	0·84	44	
44	87·40	0·88	2·06	0·45	0·12	2·25	2·63	11·27	—	1·04	39	
59	84·52	0·96	2·12	0·36	0·12	2·13	2·56	11·10	—	1·32	38	
74	81·94	0·87	2·06	0·35	0·09	2·03	2·53	10·82	—	1·58	36	
99	77·09	0·88	2·07	0·31	—	1·87	2·50	10·60	—	2·02	38	
124	72·52	0·77	1·89	0·33	—	1·66	2·34	9·89	—	2·51	46	
149	67·53	0·79	1·82	0·39	—	1·53	2·22	9·81	1 rot removed.	3·65	51	
174	63·72	0·77	1·77	0·29	0·11	1·38	2·34	9·15	—	4·27	53	
199	57·52	0·67	1·63	0·37	—	1·16	2·14	8·38	1 rot removed.	4·90	52	
234	51·43	0·55	1·42	0·33	—	0·98	1·99	7·40	1 rot removed.	5·75	49	
Samples changed from 3°C. *to* 10°C. *after* 47 *days at* 3°C.												
47 +												
5	85·78	0·94	2·15	0·25	0·09	2·14	2·55	11·18	—	—	—	—
10	84·66	0·91	2·11	0·36	—	2·12	2·49	—	—	—	—	—
15	83·67	0·92	2·06	0·32	—	2·03	2·49	—	—	—	—	—
20	82·70	0·95	2·03	0·33	—	2·02	2·56	—	—	—	—	—
29	81·08	1·02	2·08	0·20	—	1·86	2·52	—	—	—	—	—
39	78·91	0·99	2·12	0·25	—	1·79	2·59	—	—	—	—	—
49	76·40	0·97	2·06	0·25	—	1·74	2·39	—	—	—	—	—
59	74·37	0·89	2·08	0·33	0·11	1·61	2·38	10·09	—	—	—	—
Storage temperature 10°C.												
4	98·57	0·78	2·05	0·90	0·36	2·55	3·41	12·29	—	0·25	127	4·2
8	96·86	0·86	2·06	0·83	0·15	2·58	2·93	12·08	—	0·49	118	
14	95·37	0·97	2·09	0·44	0·13	2·54	2·73	11·67	—	0·79	95	
24	93·32	1·09	2·31	0·25	0·13	2·43	2·68	11·87	—	1·16	65	
34	91·87	1·02	2·32	0·31	0·12	2·29	2·63	11·75	—	1·43	53	
49	88·87	1·02	2·31	0·21	0·11	2·11	2·55	11·09	—	1·78	48	
64	86·16	0·99	2·32	0·32	—	1·97	2·53	11·11	—	2·12	49	
79	84·10	0·97	2·22	0·31	—	1·80	2·44	10·69	—	2·48	53	
94	80·63	0·94	2·13	0·28	—	1·66	2·43	10·22	1 core rot removed.	2·88	57	
109	77·62	0·77	2·11	0·33	—	1·58	2·39	9·99	1 rot removed.	3·29	60	
129	73·08	0·74	1·97	0·32	—	1·39	2·37	9·59	2 rots removed.	3·87	62	
149	67·56	0·58	1·81	0·20	—	1·25	2·37	8·99	3 rots removed.	4·46	63	
184	60·90	0·52	1·59	0·24	—	0·98	2·26	7·98	4 rots removed.	5·43	57	
219	54·00	0·41	1·25	0·17	0·10	0·80	2·18	7·14	4 rots removed.	6·33	53	
Samples changed from 10°C *to* 3°C. *after* 47 *days at* 10°C.												
47 +												
5	88·28	0·95	2·27	0·24	—	2·12	2·51	11·00	—	—	—	—
10	87·47	1·05	2·24	0·28	0·11	2·06	2·50	—	—	—	—	—
15	86·96	1·00	2·17	0·27	—	2·08	2·50	—	—	—	—	—
25	85·23	0·99	2·18	0·26	—	1·99	2·52	—	—	—	—	—
40	82·91	0·91	2·22	0·33	—	1·99	2·54	—	1 rot removed.	—	—	—
60	79·47	0·79	2·19	0·37	—	1·89	2·50	—	—	—	—	—
80	75·92	0·81	2·05	0·40	—	1·74	2·38	—	—	—	—	—
100	71·80	0·73	1·86	0·31	0·11	1·62	2·33	9·89	1 rot removed.	—	—	—
Standard deviation of 4 initial samples expressed as percentage of each constituent		3·4	2·4	4·1	3·3	0·9	1·2	1·1	—	—	—	—
Standard deviation of duplicates		2·5	2·4	3·2	—	0·4	0·4	—	—	—	—	—
At 18°C.												
0	—	—	—	—	—	—	—	—	—	—	213	3·04
5	—	—	—	—	—	—	—	—	—	—	207	
10	—	—	—	—	—	—	—	—	—	—	150	
15	—	—	—	—	—	—	—	—	—	—	111	
20	—	—	—	—	—	—	—	—	—	—	95	
25	—	—	—	—	—	—	—	—	—	—	77	
50	—	—	—	—	—	—	—	—	—	—	47	
65	—	—	—	—	—	—	—	—	—	—	70	
75	—	—	—	—	—	—	—	—	—	—	53	
100	—	—	—	—	—	—	—	—	—	—	33	
At 1°C.												
0	—	—	—	—	—	—	—	—	—	—	60	4·1
10	—	—	—	—	—	—	—	—	—	—	47	
25	—	—	—	—	—	—	—	—	—	—	43	
50	—	—	—	—	—	—	—	—	—	—	40	
75	—	—	—	—	—	—	—	—	—	—	33	
100	—	—	—	—	—	—	—	—	—	—	37	
125	—	—	—	—	—	—	—	—	—	—	40	
175	—	—	—	—	—	—	—	—	—	—	40	
200	—	—	—	—	—	—	—	—	—	—	42	

EXPERIMENT V.

Bramley's Seedling apples stored at 1°, 3°, 10°, 15°, and 20° C. in air;
also at 10° C. in N_2, O_2, gas mixture and open air.
Source: Farningham, Kent.
Gathering date 10.7.34. Number of apples per sample 40. Average weight 30 gms.

Days in store.	Relative fresh weight %.	Gm. per 100 gm. fresh weight.							Total N mg. per 100 gm.	Alcohol soluble N. mg. per 100 gm.	Rots.	Respiration		Standard error as percentage	Total volatiles mg CO_2 per 100 gm.
		Glucose.	Fructose.	Sucrose.	Starch.	Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.				Total CO_2 gm per 100 gm	Rate [illegible] per 10 K.H.		
[illegible]	98·2	1·09	1·73	0·54	0·94	2·17	4·49	8·11	89·1	34·8	—	—	—	—	—
[illegible]	98·2	1·03	1·68	0·63	0·84	2·19	4·41	8·06	83·6	30·6	—	—	—	—	—
[illegible]	98·2	1·05	1·69	0·58	0·84	2·20	4·43	8·02	87·4	33·2	—	—	—	—	—
[illegible]	98·2	1·06	1·66	0·53	0·87	2·20	4·48	8·16	84·7	30·8	—	—	—	—	—
[illegible]	98·2	1·04	1·62	0·58	0·86	2·14	4·44	8·14	88·0	32·2	—	—	—	—	—
[illegible]	98·2	0·98	1·73	0·60	0·89	2·19	4·54	8·13	84·8	30·6	—	—	—	—	—
[illegible]	98·2	1·09	1·65	0·56	0·88	2·20	4·49	8·14	85·6	31·7	—	—	—	—	—
[illegible]	98·2	1·06	1·70	0·58	0·93	2·17	4·48	8·27	84·3	32·6	—	—	—	—	—
[illegible]	98·2	1·13	1·75	0·54	0·84	2·15	4·40	8·28	85·3	32·9	—	—	—	—	—
[illegible]	98·2	1·01	1·85	0·53	0·94	2·19	4·51	8·07	87·4	32·9	—	—	—	—	—
[illegible]	98·2	1·05	1·73	0·57	0·96	2·19	4·46	8·11	85·0	32·6	—	—	—	—	—
Storage temperature 1°C. in air.															
[illegible]	98·3	1·09	1·75	0·65	0·86	2·18	4·46	7·97	—	—	—	0·01	30	4·5	—
[illegible]	98·1	1·06	1·73	0·64	0·82	2·20	4·29	8·11	—	—	—	0·03	29		—
[illegible]	97·7	1·02	1·91	0·73	0·57	2·19	4·02	8·43	—	—	—	0·06	28		—
[illegible]	97·3	1·19	1·83	0·91	0·31	2·20	3·84	8·71	—	—	—	0·10	27		—
[illegible]	96·9	1·27	1·85	0·81	0·26	2·15	3·72	8·69	—	—	—	0·16	26		—
[illegible]	96·0	1·16	2·02	0·86	0·22	2·16	3·72	8·61	—	—	—	0·20	26		—
[illegible]	96·4	1·19	1·93	0·83	0·15	2·19	3·68	8·03	—	—	—	0·24	26		—
[illegible]	96·3	1·23	1·91	0·82	0·17	2·21	3·57	8·74	—	—	—	0·29	25		—
[illegible]	95·7	1·16	2·29	0·67	0·17	2·11	3·56	8·74	—	—	—	0·44	23		—
[illegible]	95·0	1·31	2·06	0·57	0·18	2·06	3·56	8·58	—	—	—	0·58	23		—
[illegible]	93·5	1·40	2·20	0·38	0·17	1·98	3·44	8·57	—	—	—	0·74	22		—
84½	92·4	1·30	2·08	0·42	—	1·95	3·44	8·33	—	—	—	0·97	22		—
104½	92·4	1·25	2·05	0·42	—	1·81	3·41	8·25	—	—	—	1·17	23		4·7
132½	91·2	1·15	1·96	0·36	—	1·74	3·36	8·12	91·0	34·3	—	1·58	30		—
160½	90·8	1·11	2·13	0·38	—	1·62	3·25	7·86	—	—	—	1·98	33		11·4
195½	89·7	1·11	2·11	0·40	—	1·48	3·21	7·62	—	—	—	2·49	31		17·3
230½	86·6	1·12	2·10	0·37	—	1·38	3·19	7·47	—	—	—	3·00	29		24·0
307½	85·2	0·89	1·83	0·34	—	1·08	3·21	6·61	—	—	3 rots removed.	4·00	25		30·7
345½	81·9	0·87	1·78	0·25	—	0·85	3·21	6·32	—	—	—	4·43	22		45·3
Storage temperature 3°C. in air.															
[illegible]	98·3	1·09	1·69	0·58	0·78	2·21	4·41	7·94	—	—	—	0·02	54	8·0	—
[illegible]	98·0	0·97	1·83	0·64	0·77	2·21	4·31	8·13	—	—	—	0·04	61		—
[illegible]	97·5	1·02	1·75	0·85	0·58	2·20	4·11	8·22	—	—	—	0·07	46		0·6
[illegible]	97·1	1·07	1·90	0·77	0·42	2·22	3·97	8·45	—	—	—	0·10	43		—
[illegible]	96·9	1·15	1·78	0·88	0·33	2·19	3·79	8·63	—	—	—	0·14	40		1·2
[illegible]	96·7	1·18	1·85	0·84	0·22	2·24	3·79	8·78	—	—	—	0·18	38		—
[illegible]	96·5	1·11	2·02	0·70	0·23	2·16	3·75	8·90	—	—	—	0·20	36		1·8
[illegible]	96·3	1·23	1·89	0·83	0·20	2·20	3·72	8·76	—	—	—	0·25	34		—
[illegible]	96·0	1·38	2·00	0·65	0·18	2·16	3·63	8·75	—	—	—	0·40	31		3·3
[illegible]	95·1	1·37	2·22	0·53	0·18	2·08	3·51	8·65	—	—	—	0·56	30		4·7
[illegible]	95·4	1·40	2·20	0·51	0·16	2·12	3·51	9·01	—	—	—	0·78	29		6·8
[illegible]	93·2	1·42	2·11	0·41	—	1·99	3·38	8·28	—	—	—	0·95	28		8·8
[illegible]	92·8	1·38	2·10	0·33	—	1·93	3·39	8·36	—	—	—	1·18	28		13·8
[illegible]	92·1	1·31	2·12	0·33	—	1·82	3·35	8·35	83·8	35·4	—	1·42	27		20·8
[illegible]	90·6	1·31	2·14	0·37	—	1·67	3·39	8·40	—	—	—	1·80	32		34·1
[illegible]	89·2	1·29	2·14	0·33	—	1·53	3·31	8·03	—	—	—	2·04	30		48·7
[illegible]	83·8	1·17	2·06	0·39	—	1·49	3·24	7·83	—	—	—	2·80	34		72·7
[illegible]	76·8	0·97	1·95	0·38	—	1·19	3·14	6·97	—	—	1 rot removed.	4·02	30		152·0
[illegible]	72·0	0·96	1·85	0·35	—	1·08	3·17	6·80			—	4·70	29		186·0
Storage temperature 10°C. in air.															
½	98·0	1·08	1·72	0·62	0·88	2·17	4·35	8·22	—	—	—	0·02	77	3·2	—
1	97·8	1·16	1·74	0·60	0·77	2·17	4·34	8·10	—	—	—	0·03	75		—
2	97·5	1·10	1·76	0·66	0·64	2·18	4·20	8·44	—	—	—	0·07	68		—
3	97·2	1·15	1·79	0·65	0·54	2·14	4·05	8·45	—	—	—	0·10	63		—
4	97·0	1·21	1·84	0·76	0·45	2·17	3·95	8·56	—	—	—	0·13	62		0·6
6	96·6	1·15	1·84	0·80	0·31	2·21	3·72	8·68		—	—	0·20	58		—
8	96·2	1·31	1·92	0·79	0·21	2·21	3·70	8·58		—	—	0·26	54		1·2
11	95·6	1·27	2·03	0·75	0·13	2·20	3·60	8·50	—	—	—	0·36	50		1·6
17½	95·1	1·39	2·14	0·50	0·16	2·14	3·44	8·00	85·2	36·0	—	0·46	47		2·4
27½	93·9	1·49	2·21	0·31	0·16	2·02	3·34	8·87	81·7	41·8	—	0·70	42		3·8
41½	92·8	1·44	2·20	0·25	0·15	1·94	3·40	8·56	—	—	—	0·98	37		5·8
55½	91·5	1·45	2·28	0·24	—	1·81	3·37	8·52	85·8	41·0	—	1·22	33		7·8
76½	91·6	1·35	2·27	0·27	—	1·76	3·31		—	—	—	1·64	50		10·8
97½	90·5	1·25	2·25	0·37	—	1·52	3·23	8·17		—	—	2·14	54		14·3
118½	89·5	1·27	2·21	0·32	—	1·48	3·22	8·01	87·2	36·5	—	2·62	53		21·8
146½	88·1	1·18	2·14	0·30	—	1·26	3·18	7·26	—	—	1 rot removed.	3·32	49		36·5
174½	86·3	1·13	2·04	0·23	—	1·16	3·09	6·85	83·7	36·2	—	3·88	39		52·5
237½	85·7	0·96	2·03	0·21	—	0·93	3·20	6·52	82·8	36·5	—	5·02	33		88·4
272½	82·8	0·91	1·94	0·18	—	0·82	3·08	6·38	—	—	2 rots removed.	5·56	32		106·7
Storage temperature 15°C. in air															
½	97·7	1·13	1·77	0·56	0·90	2·20	4·51	8·38	—	—	—	0·02	117	5·4	—
1	97·4	1·10	1·81	0·67	0·78	2·20	4·38	8·57		—	—	0·05	111		—
2	97·0	1·12	1·79	0·66	0·75	2·27	4·26	8·73	—	—	—	0·10	103		—
3	96·6	1·12	1·74	0·75	0·55	2·19	3·98	8·58	—	—	—	0·15	98		—
4	96·2	1·24	1·85	0·67	0·40	2·15	3·62	8·61	—	—	—	0·20	95		1·8
6	95·6	1·32	1·98	0·69	0·24	2·17	3·76	8·88	—	—	—	0·30	84		—
8	95·3	1·36	2·02	0·67	0·10	2·17	3·52	8·64	—	—	—	0·40	71		2·11
10	94·8	1·48	2·05	0·44	0·15	2·15	3·51	8·73	—	—	—	0·50	70		—
16½	94·2	1·47	2·15	0·27	0·15	2·06	3·39	8·69	—	—	—	0·73	69		5·0
23½	93·6	1·49	2·21	0·18	0·16	1·93	3·30	8·56	—	—	—	0·96	63		6·3
33½	92·2	1·42	2·22	0·23	0·16	1·79	3·29	8·43	—	—	—	1·20	54		8·2
43½	90·7	1·42	2·23	0·25	—	1·61	3·28	8·15	—	—	—	1·45	48		10·6
57½	90·8	1·44	2·29	0·29		1·43	3·22	8·19	82·5	36·1	—	1·83	53		13·9
71½	88·1	1·31	2·19	0·27		1·35	3·19	7·79	—	—	—	2·19	63		17·6
85½	88·0	1·23	2·23	0·20		1·22	3·06	7·36	—	—	1 rot removed.	2·55	55		26·2
106½	87·1	1·17	2·31	0·20	—	1·11	3·12	7·40	—	—	1 rot removed.	3·09	49		41·2
127½	85·8	1·15	2·22	0·20	—	0·96	3·16	6·92	—	—	1 rot removed.	3·56	44		55·2
176½	75·0	0·88	1·91	0·12	0·13	0·63	3·12	6·02	—	—	1 rot removed.	4·48	33		83·[illegible]
204½	72·7	0·92	1·97	0·17	—	0·64	3·16	6·08	—	—	4 rots removed.	4·93	[illegible]		[illegible]
Storage temperature 20°C. in air.															
½	97·3	1·01	1·77	0·56	0·87	2·20	4·44	8·43	—	—	—	0·03	153	3·6	—
1	97·0	1·03	1·84	0·58	0·82	2·19	4·20	8·49	—	—	—	0·07	151		—
1½	96·7	1·16	1·76	0·65	0·60	2·18	4·13	8·65	—	—	—	0·10	149		—
2	96·4	1·23	1·91	0·65	0·55	2·12	4·16	8·62	—	—	—	0·13	146		—
3	96·1	1·23	1·83	0·72	0·44	2·19	4·04	8·77	—	—	—	0·20	149		—
4	95·7	1·31	1·90	0·69	0·30	2·17	3·81	8·87	—	—	—	0·26	136		—
6	95·0	1·23	2·01	0·59	0·16	2·25	3·66	9·22	—	—	—	0·39	128		—
8	94·7	1·46	2·10	0·47	0·14	2·15	3·55	9·22	—	—	—	0·52	118		—
12½	93·2	1·44	2·19	0·26	—	1·99	3·32	8·76	—	—	—	0·74	104		—
[illegible]	[illegible]	[illegible]	2·24	0·16	—	2·05	3·37	8·76	—	—	—	1·00	92		—
[illegible]	[illegible]	[illegible]	2·19	0·15	—	1·67	3·33	8·45	—	—	—	1·27	78		—
[illegible]	[illegible]	[illegible]	2·33	0·23	0·15	1·47	3·28	8·07	—	—	—	1·52	65		—
[illegible]	[illegible]	[illegible]	2·20	0·24	0·14	1·30	3·12	7·76	—	—	—	1·81	114		—
[illegible]	[illegible]	[illegible]	2·26	0·23	0·16	1·18	3·35	7·43	83·3	38·5	—	2·31	104		—
[illegible]	[illegible]	[illegible]	2·22	0·19	0·15	1·06	3·15	7·35	—	—	1 rot removed.	2·75	89		—
[illegible]	[illegible]	[illegible]	2·24	0·20	0·14	0·94	3·12	6·88	—	—	2 rots removed.	3·17	83		—
[illegible]	[illegible]	[illegible]	2·09	0·13	0·13	0·75	3·14	6·62	—	—	—	3·72	79		—
[illegible]	[illegible]	[illegible]	2·05	0·13	—	0·61	3·08	6·12	—	—	2 rots removed.	4·24	77		—
Storage temperature 10°C. in nitrogen.															
12½	95·9	1·31	2·06	0·64	0·17	2·08	3·69	8·43	—	—		0·37	38	4·3	
24½	94·2	1·41	2·10	0·44	0·14	1·99	3·60	8·90	85·4	32·5	—	0·55	32		—
[illegible]	[illegible]	[illegible]	2·19	0·47		1·90	3·36	8·62				0·74	25		
[illegible]	[illegible]	[illegible]	2·23	0·40		1·79	3·11	8·49	83·2	35·3		0·92	22		—
[illegible]	[illegible]	[illegible]	2·30	0·43	—	1·61	3·17	8·45	84·0	30·2	—	1·14	19		—
[illegible]	[illegible]	[illegible]	2·28	0·20	—	1·51	3·14	8·26				1·33	19		—
[illegible]	[illegible]	[illegible]	2·34	0·26	—	1·39	3·20	8·20	88·1	43·7	—	1·50	19		—
[illegible]	[illegible]	[illegible]	2·37	0·19		1·22	3·28	8·08				1·76	17		—
[illegible]	[illegible]	[illegible]	2·31	0·34		1·10	3·24	8·08	88·8	42·5		1·98	15		—
[illegible]	[illegible]	[illegible]	2·35	0·26		1·03	3·20	7·88			—	2·18	15		—
[illegible]	[illegible]	[illegible]	2·40	0·34	0·14	0·92	2·03	7·24	79·0	34·0		2·45	17		—
[illegible]	[illegible]	[illegible]	2·27	0·27	0·15	0·89	3·10	7·20				2·72	18		—
Storage temperature [illegible] in gas mixture [illegible]															
[illegible]	[illegible]	[illegible]	[illegible]	0·55	0·15	2·17	3·70	8·82	81·2	31·1	—	0·33	32	—	—
[illegible]	[illegible]	[illegible]	[illegible]	0·45	0·18	2·10	3·50	8·76			—	0·48	30		—
[illegible]	[illegible]	[illegible]	[illegible]	0·54	—	2·00	3·45	8·72	85·5	31·0	—	0·68	27		—
[illegible]	[illegible]	[illegible]	[illegible]	0·41	—	1·87	3·38	8·69			—	0·92	25		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]		
[illegible]	[illegible]	[illegible]	[illegible]	0·24	—	1·54	3·36	8·41			1 rot removed.	1·46	25		
[illegible]	[illegible]	[illegible]	[illegible]	0·20	—	1·42	3·36	8·18	80·5	35·3	1 rot removed.	1·76	27		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	1·25	3·35	7·72	81·2		1 rot removed.	2·08	31		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	1·12	3·22	7·30	79·0	33·4	1 rot removed.	2·40	28		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		0·96	3·25	7·14			3 rots removed.	2·84	25		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0·16	0·87	3·13	6·96	80·3	30·6		3·18	17		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0·16	0·78	3·02	6·61	—		6 rots removed.	3·44	15		
[illegible]															
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]	[illegible]	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]		—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]		—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]		—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]		—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		—
[illegible]															
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—					—	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—				—	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—					—	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—		—	—	—	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—				—	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	[illegible]			—	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		—	—	—	—
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]									

EXPERIMENT VI.

Four gatherings of Worcester Pearmain apples stored at 10° C. in air.
Source: Cottenham, Cambridge.

(a) *1st pick.*

Gathering date 18.7.35. *Number of apples per sample 30.* *Average weight 25·6 gm.*

Days in store.	Relative fresh weight gm.	Gm. per 100 gm. fresh weight												Rots.	Respiration				Standard error as percentage.
		Glucose.	Fructose.	Sucrose.	Starch.			Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.	C content of alcohol insoluble residue by combustion.	C content of alcohol soluble residue by combustion.	C content of cleared solution by combustion.		Successive Group.		Continuous Group.		
					Hydrolysis by takadiastase.	Hydrolysis by takadiastase after extraction	Hydrolysis by β amylase.								Total CO_2 gm. per 100 gm.	Rate CO_2 ccs. per 10 K.H.	Total CO_2 gm. per 100 gm.	Rate CO_2 ccs. per 10 K.H	
0	100·00	1·55	3·28	0·55	2·26	2·09	—	1·10	6·52	9·12	—	—	—	—	—	—	—	—	—
0	100·00	1·46	3·32	0·49	2·09	1·86	—	1·11	6·35	8·93	—	—	—	—	—	—	—	—	—
0	100·00	1·56	3·28	0·47	2·07	1·87	—	1·10	6·27	9·14	—	—	—	—	—	—	—	—	—
0	100·00	1·30	3·27	0·55	1·93	1·88	—	1·09	6·14	8·87	—	—	—	—	—	—	—	—	—
2	98·88	1·51	3·38	0·57	2·00	1·72	—	1·08	6·39	8·65	—	—	—	—	0·10	100	0·10	99	
4	98·20	1·52	3·39	0·59	1·73	1·66	—	1·07	6·01	8·75	—	—	—	—	0·19	90	0·19	90	
4	98·06	1·56	3·24	0·49	1·54	1·42	—	1·13	5·99	8·95	—	—	—	—	0·19	90	0·19	90	
8	97·36	1·67	3·49	0·66	1·38	1·08	—	1·08	5·55	9·34	—	—	—	—	0·35	80	0·35	[illegible]	
12	96·02	2·02	3·65	0·71	0·97	0·79	—	1·08	5·19	10·20	—	—	—	—	0·51	90	0·51	[illegible]	
20	94·59	2·31	4·16	0·73	0·23	0·10	—	1·01	4·41	10·85	—	—	—	—	0·95	126	0·88	118	1·2
20	94·50	2·28	4·35	0·69	0·26	0·10	—	1·03	4·25	11·19	—	—	—	—	0·95	126	0·88	118	
30	93·27	2·22	4·14	0·54	0·16	0·05	—	0·91	3·90	10·33	—	—	—	—	1·53	119	1·46	125	
40	89·05	2·09	4·05	0·54	0·16	0·05	—	0·84	3·97	9·98	—	—	—	—	2·11	123	2·06	129	
50	83·18	1·74	3·58	0·42	0·12	0·04	—	0·77	3·72	8·86	—	—	—	—	2·68	121	2·08	129	
60	81·20	1·47	3·27	0·30	0·17	0·04	—	0·73	3·62	8·22	—	—	—	—	3·21	104	3·26	119	
Standard deviation of 4 initial samples expressed as percentage of each constituent		8·3	0·7	9·0	6·4	5·7	—	0·7	2·5	1·5	—	—	—	—	—	—	—	—	—

(b) *2nd pick.*

Gathering date 29.7.35. *Number of apples per sample 30.* *Average weight 37·2 gm.*

Days in store.	Relative fresh weight gm.	Glucose.	Fructose.	Sucrose.	Starch: Hydrolysis by takadiastase.	Starch: Hydrolysis by takadiastase after extraction	Starch: Hydrolysis by β amylase.	Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.	C content of alcohol insoluble residue by combustion.	C content of alcohol soluble residue by combustion.	C content of cleared solution by combustion.	Rots.	Successive Group: Total CO_2 gm. per 100 gm.	Successive Group: Rate CO_2 ccs. per 10 K.H.	Continuous Group: Total CO_2 gm. per 100 gm.	Continuous Group: Rate CO_2 ccs. per 10 K.H	Standard error as percentage.
0	93·22	1·48	4·30	0·68	2·37	2·11	—	1·00	6·07	9·38	—	—	—	—	—	—	—	—	—
0	98·05	1·39	4·43	0·72	2·23	2·05	—	0·98	6·11	9·45	—	—	—	—	—	—	—	—	—
0	93·78	1·46	4·31	0·70	2·21	2·07	—	0·93	6·00	9·60	—	—	—	—	—	—	—	—	—
0	93·25	1·42	4·32	0·73	2·21	2·12	—	0·96	6·12	9·70	—	—	—	—	—	—	—	—	—
2	94·57	1·44	4·53	0·80	2·20	1·98	—	0·95	5·97	9·66	—	—	—	—	0·09	86	0·09	88	
4	96·90	1·60	4·57	0·80	2·00	1·74	—	0·95	5·66	9·95	—		—	—	0·17	75	0·17	79	
4	96·92	1·45	4·53	0·75	1·70	1·59	—	0·94	5·46	9·47	—	—		—	0·17	75	0·17	79	
8	91·86	1·70	4·65	0·79	1·20	1·21	—	0·99	5·10	10·12	—	—		—	0·30	70	0·32	75	
12	95·62	1·95	5·08	1·27	0·79	0·65	—	0·95	4·39	11·47	—	—		—	0·44	71	0·47	84	
19	94·20	2·27	5·08	1·08	0·22	0·08	—	0·89	3·62	11·44	—	—	—	3 rots removed.	0·75	114	0·80	117	2·6
19	86·30	2·04	4·91	0·86	0·19	0·07	—	0·84	3·41	10·63	—	—	—	—	0·75	114	0·80	117	
30	87·65	2·07	4·80	0·54	0·15	0·03	—	0·74	3·20	10·22	—	—	—	—	1·33	108	1·42	119	
40	90·78	1·96	5·10	0·68	0·16	0·02	—	0·67	3·43	10·81	—	—	—	1 rot removed.	1·84	105	1·99	122	
50	89·86	2·01	5·00	0·60	0·15	0·02	—	0·69	3·36	10·47	—	—	—	—	2·32	100	2·56	117	
60	91·68	2·19	4·95	0·38	0·17	0·02	—	0·69	3·38	10·17	—	—		1 rot removed.	2·77	90	3·07	100	
Standard deviation of 4 initial samples expressed as percentage of each constituent		5·2	0·7	3·1	3·4	1·6	—	3·1	4·9	1·5	—	—	—	—	—	—	—	—	—

(c) *3rd pick.*

Gathering date 15.8.35. *Number of apples per sample 30.* *Average weight 62·8 gm.*

Days in store.	Relative fresh weight gm.	Glucose.	Fructose.	Sucrose.	Starch: Hydrolysis by takadiastase.	Starch: Hydrolysis by takadiastase after extraction	Starch: Hydrolysis by β amylase.	Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.	C content of alcohol insoluble residue by combustion.	C content of alcohol soluble residue by combustion.	C content of cleared solution by combustion.	Rots.	Successive Group: Total CO_2 gm. per 100 gm.	Successive Group: Rate CO_2 ccs. per 10 K.H.	Continuous Group: Total CO_2 gm. per 100 gm.	Continuous Group: Rate CO_2 ccs. per 10 K.H	Standard error as percentage.
0	100·00	1·25	5·78	1·43	2·58	2·20	2·28	0·76	5·77	11·18	2·37	4·18	3·69	—	—	—	—	—	—
0	100·00	1·13	5·73	1·58	2·51	2·17	2·15	0·76	5·71	10·92	—	—	—	—	—	—	—	—	—
0	100·00	1·19	5·75	1·34	2·34	2·09	2·08	0·74	5·51	10·95	2·25	4·19	3·61	—	—	—	—	—	—
0	100·00	1·16	5·77	1·59	2·47	2·21	2·04	0·75	5·71	11·28	—	—	—	—	—	—	—	—	—
[illegible]																			
2	[illegible]	[illegible]	5·92	1·45	2·58	2·11	2·23	0·75	5·58	11·26	2·23	4·23	3·84	—	0·07	80	—	—	
[illegible]	[illegible]	[illegible]	6·13	1·57	2·05	1·66	1·69	0·76	5·36	—	—	—	—	—	0·19	87			
[illegible]	[illegible]	[illegible]	6·06	1·78	1·49	1·07	1·19	0·70	4·49	12·04	1·04	4·46	4·11	—	0·32	105			
[illegible]	[illegible]	[illegible]	6·18	2·05	0·90	0·65	0·62	0·71	3·92	—	—	—	—	—	0·48	116			
[illegible]	[illegible]	[illegible]	[illegible]	2·18	0·64	0·43	0·38	0·69	3·63	13·10	1·52	4·84	4·21	—	0·65	118			
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0·31	0·15	0·13	0·66	3·22	—	—	—	—	—	0·82	118			
21	[illegible]	[illegible]	[illegible]	2·09	0·26	0·08	0·07	0·64	3·21	13·17	1·37	4·87	4·50	—	1·04	116			
[illegible]	[illegible]	[illegible]	6·57	1·68	0·19	0·03	0·03	0·64	3·04	12·83	1·32	4·78	4·24	—	1·26	113			
[illegible]	[illegible]	[illegible]	6·80	1·29	0·17	0·03	0·03	0·55	3·06	12·54	1·50	4·67	4·08	—	1·42	109	—	—	
32	[illegible]	[illegible]	6·71	1·50	0·17	0·02	0·03	0·60	3·05	12·50	—	4·74	4·13	—	1·02	104			
[illegible]	[illegible]	[illegible]	6·61	1·26	—	—	—	0·59	2·99	12·31	1·30	4·52	3·99	—	1·04	100	—	—	
40	[illegible]	[illegible]	6·81	1·23	—	—	—	0·57	3·05	—	—	—	—	—	2·00	94	—	—	
44	[illegible]	2·00	6·85	0·90	—	—	—	0·52	3·02	12·32	1·31	4·67	4·11	—	2·17	88	—	—	
49	94·18	1·90	6·57	0·85	—	—	—	0·51	3·05	—	—	—	—	—	2·38	80	—	—	2·7
54	[illegible]	1·88	6·56	0·71	—	—	—	0·49	2·92	11·49	1·26	4·29	3·89	—	2·55	74	—	—	
60	[illegible]	1·91	6·67	0·64	—	—	—	0·48	3·01	—	—	—	—	—	2·70	70			
[illegible]	[illegible]	1·95	6·70	0·69	0·17	0·02	0·02	0·47	3·06	11·81	1·33	4·41	4·02	—	2·92	70			
71	[illegible]	1·95	6·36	0·67	—	—	—	0·45	3·00	—	—	—	—	—	3·12	69			
75	[illegible]	1·92	6·34	0·55	—	—	—	0·43	3·00	10·77	1·34	4·08	3·74	1 rot removed.	3·25	70			
81	91·53	1·57	6·24	0·51	—	—	—	0·41	2·96	—	—	—	—	—	3·45	70			
88	[illegible]	1·69	6·40	0·55	—	—	—	0·39	2·97	10·91	1·30	4·02	3·71	1 rot removed.	3·68	70			
95	[illegible]	1·52	6·14	0·36	—	—	—	0·39	2·93	—	—	—	—	—	3·92	70			
102	[illegible]	1·50	6·18	0·46	—	—	—	0·34	2·96	10·43	1·28	3·93	3·55	1 rot removed.	4·15	70			
[illegible]	[illegible]	1·34	5·88	0·43	—	—	—	0·34	2·90	—	—	—	—	1 rot removed.	4·39	72	—	—	
118	87·85	1·39	5·74	0·41	—	—	—	0·33	2·89	9·84	1·13	3·65	3·35	—	4·69	74	—	—	
127	[illegible]	1·35	5·72	0·43	0·17	0·02	0·02	0·32	2·99	—	—	—	—	11 rots removed.	—	—	—	—	
Storage temperature 1°C.																			
5	99·33	1·30	6·09	1·86	2·10	1·81	1·72	0·78	5·28	11·83	2·19	4·43	3·99	—	0·13	32	—	—	
11	98·84	1·36	6·08	1·93	1·55	1·19	1·19	0·73	4·71	—	—	—	—	—	0·22	34	—	—	
16	98·39	1·45	6·25	2·03	1·22	0·88	0·94	0·72	4·32	12·13	1·87	4·67	4·19	—	0·26	37	—	—	
22	98·27	1·58	6·35	2·17	0·83	0·58	0·54	0·69	4·03	—	—	—	—	—	0·36	36	—	—	
28	97·90	1·92	6·71	1·84	0·29	0·11	0·11	0·65	3·34	13·12	1·45	4·98	4·51	—	0·46	34	—	—	
34	97·16	1·77	6·67	1·92	0·48	0·25	0·25	0·69	3·63	13·19	1·58	4·96	4·32	—	0·56	35	—	—	
40	96·78	2·06	6·57	1·84	0·33	0·17	0·15	0·68	3·46	13·17	1·50	4·94	4·41	—	0·66	35	—	—	
46	96·70	2·03	6·46	1·70	0·22	0·08	0·06	0·63	3·19	—	—	—	—	—	0·76	36	—	—	
51	95·94	2·16	6·72	1·48	0·23	0·04	0·05	0·66	3·30	13·26	1·44	4·83	4·41	—	0·85	34	—	—	
56	96·10	2·08	6·75	1·39	0·19	0·03	0·03	0·60	3·16	—	—	—	—	—	0·93	34	—	—	
63	95·80	2·22	6·70	1·34	0·20	0·03	0·02	0·57	3·13	12·75	1·36	4·82	4·33	—	1·05	43	—	—	
69	95·69	2·29	6·92	1·14	0·18	0·02	0·03	0·56	3·21	—	—	—	—	—	1·14	33	—	—	3·0
76	95·50	2·17	6·74	0·99	—	—	—	0·56	3·09	12·37	1·38	4·70	4·27	—	1·25	32	—	—	
83	95·48	2·28	6·77	0·97	—	—	—	0·57	3·12	—	—	—	—	—	1·36	32	—	—	
90	95·05	2·16	7·01	0·96	—	—	—	0·53	3·09	12·71	1·36	4·85	4·34	—	1·47	32	—	—	
100	94·63	2·36	6·74	0·60	—	—	—	0·55	3·09	—	—	—	—	—	1·62	31	—	—	
111	93·33	2·23	6·93	0·72	—	—	—	0·50	3·08	12·60	1·37	4·81	4·30	—	1·78	30	—	—	
120	91·40	2·15	6·73	0·50	—	—	—	0·47	2·96	—	—	—	—	1 rot removed.	1·91	31	—	—	
140	92·98	2·31	6·80	0·32	—	—	—	0·46	3·00	12·08	1·29	4·64	4·03	—	2·20	32	—	—	
160	93·40	2·06	6·81	0·52	—	—	—	0·42	3·01	12·24	1·30	4·63	3·95	—	2·51	33	—	—	
180	90·70	2·01	6·48	0·33	—	—	—	0·41	2·89	11·36	1·24	4·37	3·84	—	2·83	35	—	—	
204	90·90	1·92	6·45	0·17	0·16	0·02	0·03	0·33	2·87	11·10	1·24	4·25	3·78	1 rot removed.	3·26	37	—	—	
Standard deviation of 4 initial samples expressed as percentage of each constituent		4·3	0·4	8·2	4·1	4·9	4·9	0·1	2·0	1·6	—	—	—	—	—	—	—	—	—
Standard deviation of duplicates		3·4	0·5	3·8	—	—	—	0·09	0·4	0·4	1·9	1·3	1·1	—	—	—	—	—	—

(d) *4th pick.*

Gathering date 2.9.35. *Number of apples per sample 30.* *Average weight 69·8 gm.*

Days in store.	Relative fresh weight gm.	Glucose.	Fructose.	Sucrose.	Starch: Hydrolysis by takadiastase.	Starch: Hydrolysis by takadiastase after extraction	Starch: Hydrolysis by β amylase.	Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.	C content of alcohol insoluble residue by combustion.	C content of alcohol soluble residue by combustion.	C content of cleared solution by combustion.	Rots.	Successive Group: Total CO_2 gm. per 100 gm.	Successive Group: Rate CO_2 ccs. per 10 K.H.	Continuous Group: Total CO_2 gm. per 100 gm.	Continuous Group: Rate CO_2 ccs. per 10 K.H	Standard error as percentage.
0	98·05	1·21	6·57	1·90	1·79	1·54	—	0·61	4·71	12·07	—	—	—	—	—	—	—	—	—
0	97·82	1·20	6·49	2·10	1·80	1·50	—	0·61	4·67	12·09	—	—	—	—	—	—	—	—	—
0	97·62	1·22	6·35	1·88	1·75	1·50	—	0·62	4·64	11·82	—	—	—	—	—	—	—	—	—
0	96·42	1·10	6·30	2·06	1·81	1·53	—	0·61	4·64	11·72	—	—	—	—	—	—	—	—	—
2	98·86	1·21	6·50	2·03	1·71	1·53	—	0·61	4·63	12·70	—	—	—	—	0·08	90	—	—	
4	95·50	1·36	6·56	2·24	1·39	1·22	—	0·61	4·18	12·51	—	—	—	—	0·17	105	—	—	
4	94·96	1·44	6·68	2·31	1·52	1·19	—	0·62	4·37	12·95	—	—	—	—	0·17	105	—	—	
8	92·92	1·38	6·60	2·47	0·72	0·60	—	0·58	3·38	13·20	—	—	—	—	0·39	122	—	—	
12	94·30	1·48	6·67	2·78	0·41	0·31	—	0·56	3·03	13·55	—	—	—	—	0·62	122	—	—	
19	91·30	1·55	7·05	2·45	0·16	0·06	—	0·53	2·71	13·61	—	—	—	3 rots removed.	1·00	107	—	—	4·9
19	93·80	1·59	6·95	2·47	0·14	0·06	—	0·52	2·66	13·36	—	—	—	—	1·00	107	—	—	
30	90·60	1·91	6·62	2·06	0·09	0·03	—	0·49	2·53	12·90	—	—	—	—	1·54	98	—	—	
40	89·26	1·78	6·68	1·50	0·10	0·04	—	0·43	2·54	12·56	—	—	—	1 rot removed.	1·98	88	—	—	
51	90·36	1·66	7·21	1·27	0·06	0·02	—	0·44	2·63	12·40	—	—	—	—	2·44	86	—	—	
60	88·37	1·72	6·93	0·87	0·09	0·02	—	0·39	2·49	11·78	—	—	—	1 rot removed.	2·79	82	—	—	
Standard deviation of 4 initial samples expressed as percentage of each constituent		4·7	1·9	5·6	1·5	1·3	—	0·9	0·7	1·5	—	—	—	—	—	—	—	—	—

EXPERIMENT VII

Bramley's Seedling apples stored at 1°, 5°, 10° and 15° C. in air and at 10° C. in ethylene.
Source: Speedgate Farm, Farningham.
Gathering date 24.9.37. Number of apples per sample 20. Average weight 135·7 gm.

Days in store.	Relative fresh weight gm.	Gm. per 100 gm. fresh weight.										Rots.	[illegible]			
															[illegible]	
		Glucose.	Fructose.	Sucrose.	Starch after extract with Kox.	Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.	C. content of alcohol insoluble residue by combustion.	C. content of alcohol soluble residue by combustion.	C. content of cleared solution by combustion.		Total CO₂, gm. per 100 gm.	[illegible]	[illegible]	[illegible]
0	98·00	0·54	4·17	2·01	2·09	1·71	4·93	10·10	2·21	3·88	3·00	—	—	—	—	—
0	98·50	0·65	4·07	1·88	2·00	1·70	4·75	9·92	2·35	3·85	2·91	—	—	—	—	—
0	98·34	0·69	4·15	1·85	1·92	1·65	4·85	9·95	2·14	3·97	2·88	—	—	—	—	—
0	98·71	0·64	4·04	1·88	2·05	1·73	4·87	9·93	2·10	4·00	3·04	—	—	—	—	—
0	98·44	0·62	4·17	1·87	1·97	1·65	4·88	9·96	2·15	4·02	2·99	—	—	—	—	—
0	98·81	0·63	4·14	1·87	2·12	1·70	4·93	9·84	2·26	3·83	2·94	—	—	—	—	—
0	98·98	0·59	4·20	1·81	2·10	1·67	4·83	10·00	2·14	4·00	2·88	—	—	—	—	—
0	98·81	0·62	4·18	1·81	2·04	1·74	4·88	9·81	2·07	4·04	2·92	—	—	—	—	—
0	98·71	0·61	4·16	1·85	1·97	1·71	4·72	9·93	2·07	4·00	2·88	—	—	—	—	—
0	98·50	0·69	4·06	1·82	2·09	1·73	4·85	9·87	2·20	4·02	2·92	—	—	—	—	—
Storage temperature 1°C.																
10	98·55	0·86	4·16	2·30	1·27	1·72	4·06	10·62	1·74	4·08	3·23	—	0·13	25	—	—
20	95·97	0·98	4·17	2·34	0·83	1·65	3·50	11·25	1·51	4·20	3·36	—	0·24	24	—	—
34	96·10	1·11	4·40	2·76	0·20	1·63	2·88	11·78	1·31	4·45	3·59	—	0·38	22	—	—
48	96·97	1·14	4·53	2·98	0·12	1·62	2·69	12·11	1·26	4·61	3·88	—	0·52	22	—	—
69	96·02	1·32	4·74	2·49	0·10	1·53	2·57	12·15	1·14	4·49	3·73	—	0·75	23	—	—
90	96·47	1·32	4·98	2·17	0·09	1·52	2·56	12·10	1·16	4·52	3·73	—	0·98	23	—	—
111	94·90	1·57	4·84	1·99	0·10	1·43	2·52	11·72	1·11	4·50	3·60	—	1·20	23	—	—
136	95·24	1·53	4·87	1·79	0·10	1·42	2·45	11·54	1·06	4·35	3·54	—	1·47	24	—	—
161	95·08	1·69	4·80	1·57	0·11	1·35	2·44	11·53	1·10	4·32	3·49	—	1·77	26	—	—
186	94·78	1·76	4·87	1·25	0·10	1·22	2·40	11·05	1·04	4·16	3·52	—	2·08	27	—	—
Storage temperature 5°C.																
7	98·79	0·95	4·33	2·34	1·22	1·67	3·90	10·67	1·65	4·20	3·31	—	0·12	35	—	—
17	97·97	1·08	4·31	2·73	0·51	1·67	3·14	11·34	1·30	4·63	3·47	—	0·27	27	—	—
27	97·47	1·19	4·56	2·94	0·16	1·62	2·77	11·98	1·15	4·73	3·77	—	0·39	25	—	—
41	97·18	1·50	4·57	2·51	0·11	1·54	2·59	12·15	1·05	4·69	3·72	—	0·58	33	—	—
55	96·90	1·69	4·57	2·14	0·10	1·50	2·54	11·77	1·09	4·64	3·71	—	0·82	40	—	—
69	96·15	1·46	4·84	2·08	0·11	1·54	2·56	11·53	1·08	4·51	3·57	—	1·11	46	—	—
83	95·97	1·63	5·08	1·69	0·10	1·40	2·46	11·54	1·06	4·48	3·60	—	1·38	43	—	—
104	95·29	1·75	4·93	1·43	0·10	1·36	2·48	11·20	1·09	4·48	3·52	—	1·77	42	—	—
125	94·25	1·67	4·96	1·09	0·09	1·23	2·44	10·88	0·98	4·04	3·35	—	2·18	40	—	—
146	93·85	1·82	5·07	0·95	0·10	1·16	2·33	10·58	0·96	4·06	3·27	—	2·58	39	—	—
Storage temperature 10°C.																
7	98·37	0·95	4·31	2·28	1·18	1·70	3·84	11·10	1·69	4·51	3·54	—	0·17	42	0·17	51
14	97·25	1·15	4·46	2·68	0·49	1·71	3·17	11·77	1·37	4·65	3·73	—	0·30	38	0·32	44
24	96·15	1·36	4·55	2·62	0·19	1·73	2·67	12·14	1·21	4·76	3·64	—	0·47	42	0·55	52
34	96·78	1·47	4·75	2·29	0·11	1·58	2·57	11·79	1·14	4·63	3·72	—	0·67	54	0·82	56
44	95·22	1·51	4·90	1·84	0·11	1·50	2·47	11·66	1·11	4·58	3·60	—	0·94	62	1·13	62
54	95·30	1·45	4·95	1·92	0·11	1·40	2·45	11·46	1·07	4·52	3·58	—	1·23	60	1·42	59
68	94·10	1·55	4·97	1·42	0·11	1·33	2·40	10·92	1·05	4·34	3·43	—	1·62	58	1·80	56
82	93·53	1·87	4·99	1·09	0·11	1·24	2·38	10·82	1·02	4·29	3·42	—	2·00	54	2·12	54
96	92·00	1·69	5·18	1·04	0·11	1·19	2·40	10·85	1·10	4·26	3·54	—	2·34	50	2·49	53
117	89·94	1·83	4·95	0·67	0·11	1·09	2·38	10·28	1·11	4·04	3·37	—	2·84	45	—	—
Storage temperature 15°C.																
5	98·30	0·76	4·48	2·05	1·51	1·71	4·26	10·64	1·95	4·21	3·24	—	0·15	56	—	—
12	96·58	1·28	4·65	2·28	0·44	1·68	2·99	11·66	1·36	4·55	3·60	—	0·34	58	—	—
19	95·96	1·46	4·87	2·19	0·13	1·52	2·47	11·64	1·13	4·60	3·74	1 small rot cut out.	0·56	50	—	—
26	95·70	1·59	4·86	1·94	0·16	1·49	2·49	11·57	1·09	4·57	3·74	—	0·88	116	—	—
33	95·65	1·81	5·13	1·43	0·16	1·42	2·43	11·42	1·09	4·53	3·58	1 small rot cut out.	1·26	114	—	—
43	94·13	1·73	5·08	1·05	0·16	1·33	2·41	11·02	1·04	4·25	3·39	—	1·78	110	—	—
53	92·92	1·79	5·08	0·47	0·13	1·05	2·35	10·44	1·03	4·04	3·21	5 rots removed.	2·29	107	—	—
63	95·99	1·86	5·33	0·89	0·14	1·23	2·41	11·13	1·10	4·38	3·48	1 rot removed.	2·80	108	—	—
77	91·65	1·79	5·02	0·44	0·11	0·84	2·25	9·68	1·03	3·88	3·22	8 rots removed.	3·53	114	—	—
91	93·80	1·70	5·19	0·68	0·15	1·12	2·35	10·27	1·05	4·19	3·28	1 rot removed.	—	—	—	—
Storage temperature 10°C. *in air containing* 1 *in* 500 *of ethylene.*																
7	97·37	0·93	4·33	2·36	1·11	1·64	3·78	11·61	1·48	4·29	3·22	—	0·19	65	—	—
14	97·00	0·90	4·59	2·78	0·44	1·62	2·91	12·22	1·27	4·45	3·52	—	0·40	64	—	—
24	96·20	1·28	4·42	2·87	0·18	1·61	2·60	12·54	1·13	4·67	3·71	1 rot removed.	0·70	64	—	—
34	97·38	1·45	4·65	2·33	0·11	1·52	2·49	12·04	1·13	4·66	3·63	1 rot removed.	1·01	66	—	—
44	96·22	1·48	4·95	1·96	0·12	1·49	2·47	11·77	1·10	4·63	3·72	—	1·33	65	—	—
54	95·36	1·55	5·09	1·59	0·11	1·32	2·44	11·36	1·09	4·36	3·47	—	1·64	65	—	—
68	95·24	1·58	5·03	1·34	0·11	1·37	2·42	11·05	1·02	4·19	3·34	1 rot removed.	2·07	63	—	—
82	94·28	1·84	4·96	1·01	0·09	1·23	2·34	10·56	1·03	4·18	3·35	—	2·48	60	—	—
96	92·49	1·63	4·93	0·86	0·11	1·21	2·37	10·28	1·03	4·14	3·35	—	2·86	56	—	—
117	93·38	1·47	4·87	0·74	0·10	1·11	2·32	10·27	1·03	3·94	3·33	—	—	—	—	—
Storage temperature 10°C. *in air for* 14 *days, and then in air containing* 1 *in* 500 *ethylene.*																
7	97·88	0·92	4·30	2·22	1·14	1·65	3·76	11 46	1·63	4·43	3·64	—	0·16	43	—	—
14	97·72	1·20	4·25	2·57	0·45	1·67	3·02	11 68	1·29	4·57	3·76	—	0·30	40	—	—
24	97·18	1·29	4·51	2·31	0·18	1·58	2·55	11 68	1·08	4·51	3·85	—	0·52	56	—	—
34	95·13	1·43	4·70	1·97	0·12	1·52	2·51	11·87	1·07	4·49	3·51	—	0·79	63	—	—
44	95·92	1·10	5·23	1·92	0·13	1·51	2·52	11 71	1·08	4·46	3·60	—	1·08	61	—	—
54	95·15	1·46	4·86	1·74	0·12	1·45	2·49	11·44	1·08	4·44	3·77	—	1·36	60	—	—
68	94·82	0·91	5·20	1·80	0·10	1·39	2·42	10 91	1·01	4·21	3·44	—	1·77	61	—	—
82	92·82	1·66	5·28	1·03	0·11	1·30	2·44	11·19	1·06	4·12	3·41	—	2·20	68	—	—
96	93·78	1·63	4·92	0·96	0·10	1·24	2·38	10 47	1·02	4·11	3·28	1 rot removed.	2·64	65	—	—
117	92·84	1·65	4·95	0·67	0·10	1·01	2·32	10·15	0·97	4·01	3·36	—	—	—	—	—
Standard deviation of 10 initial samples, expressed as percentage of each constituent		7·2	1·4	3·1	3·3	1·8	1·4	0·8	4·0	1·9	1·9	—	—	—	—	—
Standard deviation of duplicate samples		7·9	1·4	6·8	—	0·9	0·8	—	0·5	2·0	2·0	—	—	—	—	—

Experiment VII.

Bramley's Seedling apples stored at 1°, 5°, 10° and 15° C. in air and at 10° C. in ethylene.

Source: Speedgate Farm, Farningham.

Gathering date 24.9.37. Number of apples per sample 20. Average weight 135·7 gm.

		Gm. per 100 gm. fresh weight.												[illegible]		
															[illegible]	
Days in store.	Relative fresh weight gm.	Glucose.	Fructose.	Sucrose.	Starch after extract with Kox.	Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.	C. content of alcohol insoluble residue by combustion.	C. content of alcohol soluble residue by combustion.	C. content of cleared solution by combustion.	Rots.	Total CO₂ gm. per 100 gm.	[illegible]	[illegible]	[illegible]
0	98·60	0·54	4·17	2·01	2·09	1·71	4·93	10·10	2·21	3·88	3·00	—	—	—	—	—
0	98·50	0·65	4·07	1·88	2·00	1·70	4·75	9·92	2·35	3·85	2·91	—	—	—	—	—
0	98·34	0·69	4·15	1·85	1·92	1·65	4·85	9·95	2·14	3·97	2·88	—	—	—	—	—
0	98·71	0·64	4·04	1·88	2·05	1·73	4·87	9·93	2·10	4·00	3·04	—	—	—	—	—
0	98·44	0·62	4·17	1·87	1·97	1·65	4·88	9·96	2·15	4·02	2·99	—	—	—	—	—
0	98·81	0·63	4·14	1·87	2·12	1·70	4·93	9·84	2·26	3·83	2·94	—	—	—	—	—
0	98·98	0·59	4·20	1·81	2·10	1·67	4·83	10·00	2·14	4·00	2·88	—	—	—	—	—
0	98·81	0·62	4·18	1·81	2·04	1·74	4·88	9·81	2·07	4·04	2·92	—	—	—	—	—
0	98·71	0·61	4·16	1·85	1·97	1·71	4·72	9·93	2·07	4·00	2·88	—	—	—	—	—
0	98·50	0·69	4·06	1·82	2·09	1·73	4·85	9·87	2·20	4·02	2·92	—	—	—	—	—
Storage temperature 1°C.																
10	98·55	0·86	4·16	2·30	1·27	1·72	4·06	10·62	1·74	4·08	3·23	—	0·13	25	—	—
20	95·97	0·98	4·17	2·34	0·83	1·65	3·50	11·25	1·51	4·20	3·36	—	0·24	24	—	—
34	96·10	1·11	4·40	2·76	0·20	1·63	2·88	11·78	1·31	4·45	3·59	—	0·38	22	—	—
48	96·97	1·14	4·53	2·98	0·12	1·62	2·69	12·11	1·26	4·61	3·88	—	0·52	22	—	—
69	96·02	1·32	4·74	2·49	0·10	1·53	2·57	12·15	1·14	4·49	3·73	—	0·75	23	—	—
90	96·47	1·32	4·98	2·17	0·09	1·52	2·56	12·10	1·16	4·52	3·73	—	0·98	23	—	—
111	94·90	1·57	4·84	1·99	0·10	1·43	2·52	11·72	1·11	4·50	3·60	—	1·20	23	—	—
136	95·24	1·53	4·87	1·79	0·10	1·42	2·45	11·54	1·06	4·35	3·54	—	1·47	24	—	—
161	95·08	1·69	4·80	1·57	0·11	1·35	2·44	11·53	1·10	4·32	3·49	—	1·77	26	—	—
186	94·78	1·76	4·87	1·25	0·10	1·22	2·40	11·05	1·04	4·16	3·52	—	2·08	27	—	—
Storage temperature 5°C.																
7	98·79	0·95	4·33	2·34	1·22	1·67	3·90	10·67	1·65	4·20	3·31	—	0·12	35	—	—
17	97·97	1·08	4·31	2·73	0·51	1·67	3·14	11·34	1·30	4·63	3·47	—	0·27	27	—	—
27	97·47	1·19	4·56	2·94	0·16	1·62	2·77	11·98	1·15	4·73	3·77	—	0·39	25	—	—
41	97·18	1·50	4·57	2·51	0·11	1·54	2·59	12·15	1·05	4·69	3·72	—	0·58	33	—	—
55	96·90	1·69	4·57	2·14	0·10	1·50	2·54	11·77	1·09	4·64	3·71	—	0·82	40	—	—
69	96·15	1·46	4·84	2·08	0·11	1·54	2·56	11·53	1·08	4·51	3·57	—	1·11	46	—	—
83	95·97	1·63	5·08	1·69	0·10	1·40	2·46	11·54	1·06	4·48	3·60	—	1·38	43	—	—
104	95·29	1·75	4·93	1·43	0·10	1·36	2·48	11·20	1·09	4·48	3·52	—	1·77	42	—	—
125	94·25	1·67	4·96	1·09	0·09	1·23	2·44	10·88	0·98	4·04	3·35	—	2·18	40	—	—
146	93·85	1·82	5·07	0·95	0·10	1·16	2·33	10·58	0·96	4·06	3·27	—	2·58	39	—	—
Storage temperature 10°C.																
7	98·37	0·95	4·31	2·28	1·18	1·70	3·84	11·10	1·69	4·51	3·54	—	0·17	42	0·17	51
14	97·25	1·15	4·46	2·68	0·49	1·71	3·17	11·77	1·37	4·65	3·73	—	0·30	38	0·32	44
24	96·15	1·36	4·55	2·62	0·19	1·73	2·67	12·14	1·21	4·76	3·64	—	0·47	42	0·55	52
34	96·78	1·47	4·75	2·29	0·11	1·58	2·57	11·79	1·14	4·63	3·72	—	0·67	54	0·82	56
44	95·22	1·51	4·90	1·84	0·11	1·50	2·47	11·66	1·11	4·58	3·60	—	0·94	62	1·13	62
54	95·30	1·45	4·95	1·92	0·11	1·40	2·45	11·46	1·07	4·52	3·58	—	1·23	60	1·42	59
68	94·10	1·55	4·97	1·42	0·11	1·33	2·40	10·92	1·05	4·34	3·43	—	1·62	58	1·80	56
82	93·53	1·87	4·99	1·09	0·11	1·24	2·38	10·82	1·02	4·29	3·42	—	2·00	54	2·12	54
96	92·00	1·69	5·18	1·04	0·11	1·19	2·40	10·85	1·10	4·26	3·54	—	2·34	50	2·49	53
117	89·94	1·83	4·95	0·67	0·11	1·09	2·38	10·28	1·11	4·04	3·37	—	2·84	45	—	—
Storage temperature 15°C.																
5	98·30	0·76	4·48	2·05	1·51	1·71	4·26	10·64	1·95	4·21	3·24	—	0·15	56	—	—
12	96·58	1·28	4·65	2·28	0·44	1·68	2·99	11·66	1·36	4·55	3·60	—	0·34	58	—	—
19	95·96	1·46	4·87	2·19	0·13	1·52	2·47	11·64	1·13	4·60	3·74	1 small rot cut out.	0·56	50	—	—
26	95·70	1·59	4·86	1·94	0·16	1·49	2·49	11·57	1·09	4·57	3·74	—	0·88	116	—	—
33	95·65	1·81	5·13	1·43	0·16	1·42	2·43	11·42	1·09	4·53	3·58	1 small rot cut out.	1·26	114	—	—
43	94·13	1·73	5·08	1·05	0·16	1·33	2·41	11·02	1·04	4·25	3·39	—	1·78	110	—	—
53	92·92	1·79	5·08	0·47	0·13	1·05	2·35	10·44	1·03	4·04	3·21	5 rots removed.	2·29	107	—	—
63	95·99	1·86	5·33	0·89	0·14	1·23	2·41	11·13	1·10	4·38	3·48	1 rot removed.	2·80	108	—	—
77	91·65	1·79	5·02	0·44	0·11	0·84	2·25	9·68	1·03	3·88	3·22	8 rots removed.	3·53	114	—	—
91	93·80	1·70	5·19	0·68	0·15	1·12	2·35	10·27	1·05	4·19	3·28	1 rot removed.	—	—	—	—
Storage temperature 10°C. in air containing 1 in 500 of ethylene.																
7	97·37	0·93	4·33	2·36	1·11	1·64	3·78	11·61	1·48	4·29	3·22	—	0·19	65	—	—
14	97·00	0·90	4·59	2·78	0·44	1·62	2·91	12·22	1·27	4·45	3·52	—	0·40	64	—	—
24	96·20	1·28	4·42	2·87	0·18	1·61	2·60	12·54	1·13	4·67	3·71	1 rot removed.	0·70	64	—	—
34	97·38	1·45	4·65	2·33	0·11	1·52	2·49	12·04	1·13	4·66	3·63	1 rot removed.	1·01	66	—	—
44	96·22	1·48	4·95	1·96	0·12	1·49	2·47	11·77	1·10	4·63	3·72	—	1·33	65	—	—
54	95·36	1·55	5·09	1·59	0·11	1·32	2·44	11·36	1·09	4·36	3·47	—	1·64	65	—	—
68	95·24	1·58	5·03	1·34	0·11	1·37	2·42	11·05	1·02	4·19	3·34	1 rot removed.	2·07	63	—	—
82	94·28	1·84	4·96	1·01	0·09	1·23	2·34	10·56	1·03	4·18	3·35	—	2·48	60	—	—
96	92·49	1·63	4·93	0·86	0·11	1·21	2·37	10·28	1·03	4·14	3·35	—	2·86	56	—	—
117	93·38	1·47	4·87	0·74	0·10	1·11	2·32	10·27	1·03	3·94	3·33	—	—	—	—	—
Storage temperature 10°C. in air for 14 days, and then in air containing 1 in 500 ethylene.																
7	97·88	0·92	4·30	2·22	1·14	1·65	3·76	11 46	1·63	4·43	3·64	—	0·16	43	—	—
14	97·72	1·20	4·25	2·57	0·45	1·67	3·02	11 68	1·29	4·57	3·76	—	0·30	40	—	—
24	97·18	1·29	4·51	2·31	0·18	1·58	2·55	11 68	1·08	4·51	3·85	—	0·52	56	—	—
34	95·13	1·43	4·70	1·97	0·12	1·52	2·51	11·87	1·07	4·49	3·51	—	0·79	63	—	—
44	95·92	1·10	5·23	1·92	0·13	1·51	2·52	11 71	1·08	4·46	3·60	—	1·08	61	—	—
54	95·15	1·46	4·86	1·74	0·12	1·45	2·49	11 44	1·08	4·44	3·77	—	1·36	60	—	—
68	94·82	0·91	5·20	1·80	0·10	1·39	2·42	10 91	1·01	4·21	3·44	—	1·77	61	—	—
82	92·82	1·66	5·28	1·03	0·11	1·30	2·44	11 19	1·06	4·12	3·41	—	2·20	68	—	—
96	93·78	1·63	4·92	0·96	0·10	1·24	2·38	10 47	1·02	4·11	3·28	1 rot removed.	2·64	65	—	—
117	92·84	1·65	4·95	0·67	0·10	1·01	2·32	10·15	0·97	4·01	3·36	—	—	—	—	—
Standard deviation of 10 initial samples expressed as percentage of each constituent		7·2	1·4	3·1	3·3	1·8	1·4	0·8	4·0	1·9	1·9	—	—	—	—	—
Standard deviation of duplicate samples . .		7·0	1·4	0·8	—	0·9	0·8	—	0·5	2·0	2·0	—		—	—	

SECTION II.

By A. C. HULME.

The results recorded in this section form part of a programme of work which was started in 1931. The nitrogen metabolism of the apple had at that time been very little studied thus leaving a serious gap in the data available for the formation of a coherent theory of the general metabolism of the apple fruit. As the work progressed it became clear that in the developing apple respiration-rate was closely linked with protein metabolism. It therefore became advisable to record the respiration-rate and, as an obvious next step, the content of the more important carbohydrates when making determinations of the nitrogenous constituents of samples of apples. This procedure was adopted in the later experiments recorded in this section.

The chief aim of the experiments herein recorded was to establish the general trend of the metabolism of nitrogen during the development of an apple on the tree, and when gathered and stored at various stages of development, as a fundamental step in the study of storage behaviour.

Bramley's Seedling apples were used except in one experiment for which Early Victoria apples were chosen as an example of an early, or summer, variety.

MATERIAL AND METHODS OF SAMPLING.

Bramley's Seedling. All the fruit used from 1936 to 1939 was from the same trees and a description of the material and of the methods of sampling has already been published (I.—under Series MBY/34). In this paper also the method used in the preparation of the material for analysis is described.

The Bramley's Seedling apples used in 1945 were from 6 trees on Malling II stocks. Sampling was by the usual method. Respiration data only are given for these samples. This experiment was designed to determine changes in respiration-rate, nitrogen content and sugar content within the first few hours of gathering, and will be described in detail elsewhere.

Early Victoria. These apples were from the same orchard as the Bramley's Seedling apples used in 1936-39. The same random methods of sampling, etc., were used as for the Bramley's Seedling apples.

The treatment of fruit not for immediate analysis (i.e. all samples other than initial samples) will be described later.

Time in days either dates from Petal Fall (developing fruit) or from the time the fruit was placed in storage at a constant temperature (storage samples). The dates taken as " Petal Fall " for the fruit under review were as follows :

1936. Bramley's Seedling : 21st May, 1936.
1938. Bramley's Seedling : 12th May, 1938.
1939. Bramley's Seedling : 22nd May, 1939.
1939. Early Victoria : 22nd May, 1939.
1945. Bramley's Seedling : 26th April, 1945.

Records of orchard temperature throughout the picking season are available but are not included in this report.

PREPARATION OF SAMPLES FOR ANALYSIS.

The method used was essentially that described in Section I of this report. Details are also given elsewhere (1).

LOSS OF WEIGHT DURING STORAGE

Since all the storage samples were stored in desiccators for the measurement of respiration-rate, weight-loss was obtained by weighing the samples when removed from these desiccators for analysis. Where rotting occurred, rotted fruits were removed from time to time so that they should not introduce errors into the measurement of respiration. These rotten apples were weighed and corrections applied so that the data for weight loss apply, as far as possible, to sound fruit only. Where small rotten areas were noted when the fruit was removed for analysis these small areas were cut out before the fruit was frozen to await analysis.

METHODS OF ANALYSIS.

DETERMINATION OF TOTAL NITROGEN.

This determination was carried out by the method described by Hulme (1) in 1936. In 1937 the use of selenium as a catalyst was discontinued and 5 ml. of 100 vol. hydrogen peroxide were added when the digest was dark brown but transparent, to shorten the time of digestion.

PREPARATION OF THE EXTRACT FOR THE DETERMINATION OF NON-PROTEIN NITROGEN AND ITS FRACTIONS, SUGARS AND ACID.

The extract was prepared by extraction of an aliquot of frozen, ground and mixed tissue with 85 per cent. alcohol *in vacuo* as already described (1) but using the rapid continuous vacuum extractor described by Hulme and Roach (2). In the case of the Bramley's Seedling apples in 1939, the fractions of the soluble nitrogen were not determined, 100 g. of tissue only were used and extraction was carried out in an all-glass modification of the continuous vacuum extractor.

The extracted residues of the tissue were carefully dried *in vacuo* at 55-60° C. These residues were quite white in colour. They were ground to a fine powder and designated " alcohol-insoluble residue ". The alcohol extracts were evaporated *in vacuo* to a small volume (60-100 ml.) and this solution was used for the determination of soluble nitrogen and its fractions, sugar and acid by the methods to be described ; it will be referred to hereafter as " the extract ".

Protein nitrogen was determined in aliquot portions of the well-mixed alcohol-insoluble residue by the micro-Kjeldahl technique described elsewhere (1, p. 262). In many cases this fraction was obtained merely by subtracting total soluble nitrogen from the total nitrogen of the tissue.

Free ammonia and amide-nitrogen were determined by the method described in the above-mentioned paper (1, p. 262).

Amino nitrogen was determined by the method of Hulme (3).

Amino-acid nitrogen = amino N − amide N.

" *Rest* " *nitrogen* = total soluble nitrogen—(free ammonia N+*asparagine nitrogen+amino acid N).

Titratable acid was also determined on an aliquot portion of the extract by the method already described (1).

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* For the reasons given by Hulme (1), asparagine N = amide N × 2.

SUGARS.

For the estimation of sugars an aliquot portion of the extract was neutralized and cleared with basic lead acetate, filtered and the excess lead removed by potassium oxalate. The solution was again filtered and made up to a known volume of water. Preliminary tests were made to determine the amount of lead acetate and potassium oxalate necessary in each case to avoid an undue excess of each reagent.

Glucose+fructose was estimated in the cleared extract by a modification of the Somolgi copper reduction method developed by C. S. Hanes.* The copper reagent used (Hanes's "sodium sulphate I") is made up as follows:

Sodium carbonate	30 gm.	Dissolve in 750 ml. water.
Rochelle salt	25 gm.	
Copper sulphate ($5H_2O$)	7·5 gm.	Add in 50 ml. water.
Anhydrous sodium sulphate	160 gm.	Add dry.
Potassium iodide	1·5 gm.	,, ,,
Sodium bicarbonate	17 gm.	,, ,,

The whole is then brought to the boil and cooled. 0·75 gm. of potassium iodate is then added and the solution made up to 1 litre with water. To prevent crystallization of sodium sulphate the solution is held at 25° C.

5 ml. of this reagent together with 5 ml. of the cleared extract (diluted if necessary) containing up to 1·8 mg. of reducing sugar were heated for 15 minutes at 100° C. in tubes fitted with air condensers. The tubes were then cooled for 2 minutes in running water, 2 ml. of a solution containing 2·5 per cent. potassium iodide and 2·5 per cent. potassium oxalate (5 per cent. stock solutions of these reagents were mixed every 10 days), followed by 3 ml. of 2N sulphuric acid were added and the contents well mixed. The tubes must then be allowed to stand for at least 10 minutes before titration of the liberated iodine with N/75 sodium thiosulphate. A blank titration was carried out using 5 ml. of water in place of the extract (sugar-solution). This blank should be between 7·5 and 8·0 ml. thiosulphate. The amount of copper reduced and hence the amount of sugar present was obtained by subtraction of the thiosulphate figure of the test-solution from that of the water-blank. 1 ml. of N/100 thiosulphate was found to be equivalent to 0·225 mg. of glucose and of invert sugar over the range 0 to 2 mg. sugar. All the figures for sugar in the data tables were calculated from this value.

Sucrose was determined after inversion with N.HCl (50 ml. extract plus 5 ml. of 5·5 N.HCl) for 30 minutes at 60° C. The inversion was carried out in stoppered conical flasks attached to a bar moving to and fro in the constant-temperature bath to keep the liquids stirred. After cooling and neutralizing to phenol red, the invert sugar was determined on 5 ml. aliquots of the inversion liquid by the method of Hanes described above.

DETERMINATION OF STARCH.

Starch was determined on the alcohol-insoluble residues by measuring the intensity of the blue colour of the starch-iodide compound developed under carefully standardized conditions.

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* Since this method, which in the form used was due to Dr. Hanes, has not been published it is necessary to give essential details here.

(i) *Extraction of starch from the alcohol-insoluble residues.*

The following technique was developed from that described by Hanes (5) to give a rapid, accurate routine method which obviated the necessity for filtration at any stage in the preparation of the starch extract.

0·1 to 0·2 gm. of alcohol-insoluble residue was weighed into 50 ml. centrifuge tubes the top of which incorporated a B24 standard ground glass socket to which could be fitted a short glass air-condenser via a two-way all-glass adapter. The starch was rendered soluble by treatment with alcohol containing 1 per cent. HCl as follows: 20 ml. of alcohol were added to the centrifuge tubes and the air condenser placed in one arm of the glass adapter, the other arm being fitted with a ground-glass stopper. The centrifuge tube was placed in a boiling water-bath and, when the alcohol was boiling, the glass stopper in the adapter was removed, 0·2 ml. HCl rapidly added and the stopper replaced. Boiling was continued for exactly 12 minutes and the tube was then rapidly cooled. After centrifugation, the supernatant liquid was discarded and the residue washed three times with 20 ml. of 95 per cent. alcohol, each lot of wash-liquid being centrifuged off. Tests showed that three washings were sufficient to remove all the HCl from the extract.

The next stage was the quantitative extraction of the soluble starch from the residue. 20 ml. of boiling distilled water were added to the residue from which practically all the alcohol had been removed by the last centrifugation. The contents of the tube were then gently boiled over a bare flame for 2 minutes, care being taken to avoid spitting. The air condensers were then fitted to the tube (the adapter being no longer required) and the tubes placed in a boiling water bath for 20 minutes. The tubes were then cooled and the supernatant liquid carefully poured into a standard flask. The residue was boiled with 10 ml. water and, after cooling, the entire contents of the tube were washed into the standard flask and the liquid brought to the mark with distilled water. The residue was then filtered off on a small Buchner funnel and the filtrate placed in glass stoppered bottles to await the starch determination. The bulk of the residue was so small that no volume correction was necessary.

(ii) *Preparation of the starch-iodide compound.*

It was found by experience that, over the range of 0 to 5 mg. ml. of starch in the final extract, the best concentration of iodine was 5 per cent. of a solution of 0·2 gm. iodine+0·3 gm. potassium iodide in the final solution used for colour-matching.

A suitable aliquot of the starch extract (usually 5 ml.) was transferred to a 50 ml. volumetric flask, and about 25 ml. of water added, followed by 2·5 ml. of the iodine solution. The flask was shaken well and water added to the 50 ml. mark. Determinations of the intensity of the starch-iodide blue colour must be made on this solution within a few hours of its preparation.

(iii) *Determination of the concentration of starch-iodide compound.*

In preliminary experiments the intensity of the starch-iodide blue colour was determined and the percentage of starch in the original residue calculated therefrom, by matching against (a) starch solutions prepared directly from a sample of apple-starch kindly provided by Dr. Hanes, and (b) starch solutions prepared from starch-free alcohol-insoluble residues obtained from the same series of storage experiments as the ones under investigation plus known amounts of added apple-starch, in an ordinary colorimeter, using micro-cups and a sodium lamp as the source of illumination. Standards (a) and (b) gave slightly different results. Standard (b) was therefore used since its method of preparation corresponds closely with that used in preparing extracts of starch from samples of tissue under examination. Later it was found possible by the use of suitable filters, to calibrate the colorimeter (Duboscq type) in " starch units " and so obviate the necessity of preparing standard starch solutions for each series of determinations. This procedure virtually converts the colorimeter into a photometer. An Ilford Spectrum Yellow Filter No. 606 (transmits between 5,600 Å and 6,100 Å) was fitted over the eyepiece, and an Ilford Neutral Filter, density 0·5, was arranged to cover the source of illumination of the left-hand cup of a Duboscq colorimeter by means of a special holder fitted to the normal colorimeter-lamp of the instrument. A 24-watt, 12-volt bulb was used in the lampholder. With this arrangement the instrument was calibrated with extracts of apple-starch obtained by adding known amounts of the starch to starch-free alcohol residues and treating exactly as described above. The final standard starch-iodide solutions corresponding to a range of original starch contents were placed in the right-hand micro-cup of the colorimeter. The left-hand cup contained distilled water only, and the vernier corresponding to this cup was always set at " 15 ". It was found that the results followed de Beer's law, the reciprocal of the vernier reading of the left-hand cup being a linear function of the quantity of starch per ml. of solution in the right hand cup. The line expressing this relationship did not, however, pass through the origin. The values of a and b in the equation $y = a+bx$, where y represents the reciprocal of the reading of the right hand vernier and x represents the mg. of starch per ml. in the solution in the right hand cup, were calculated by the method of least squares from a series of results using starch concentrations between 0·01 and 0·1 mg./ml. These values were found to be $a=0{\cdot}0092$ and $b=1{\cdot}77$. Hence mg. starch per ml. $=\dfrac{1/\mathrm{R}-0{\cdot}0092}{1{\cdot}77}$, where R represents the vernier reading corresponding to the right hand cup.

The results obtained for starch, using the modified colorimeter, were checked at several points by measuring the extinction coefficients of a selection of the starch-iodide solutions in the cell of a Zeiss-Pulfrich Photometer (using Filter No. 15).

Wheat starch, added to starch-free residues and extracted in the usual way, also gave a linear relationship between mg. starch/ml. and 1/R, but the constants " a " and " b " were slightly different being respectively, 0·007 and 1·57.

The factors derived from apple starch were used in calculating starch-content in all the results recorded in this report.

DETERMINATION OF THE CO_2 OF RESPIRATION.

The samples of fruit used in the study of metabolic changes occurring during storage were gathered and treated prior to storage in exactly the same way as the initial samples. At the same time as the initial samples were frozen to −20° C. to await analysis, the corresponding series of samples were placed in 5-6 litre desiccators which formed part of a train of apparatus for measuring the CO_2 evolved by the apples. This apparatus and the method of absorbing and estimating the CO_2 evolved have been described elsewhere (4). An apparatus for measuring the temperature of a sample of fruit was run alongside each series for the first 48 hours. Thermocouples were inserted into several of the fruits, the leads from the thermocouples being sealed by wax into a tube in the tube in the bung of the desiccator. This procedure was adopted to ascertain the point at which the fruit attained the temperature of the storage chamber. The time taken to attain this temperature varied with the temperature of the fruit when harvested and also with the size of the fruit. The longest time taken was 12-14 hours. Due allowance was made in the data for rate of respiration given in the tables for the period taken for the fruit to attain the correct temperature.

A close watch was kept on the fruit during respiration-measurements and as soon as an apple was observed to be attacked by a rot the desiccator was opened, the rotting apple removed and weighed and due correction made in subsequent determinations of the CO_2-output of the sample.

DETERMINATION OF DRY WEIGHT.

The dry weight of a sample was determined, in duplicate, by weighing 50 g. aliquot portions of the frozen and ground tissue into beakers in the —20° C. room and then drying *in vacuo* over calcium chloride at 50° C. for 48 hours. The dry weight was then found by weighing to the nearest 10 mg. This method was found to give consistent results which approximated closely to those obtained by drying to constant-weight *in vacuo* over P_2O_5 at 25° C.

EXPRESSION OF RESULTS.

(i) *General.*

The standard of reference chosen throughout this section has been the unitary growing organ—the growing apple on the tree or the " shrinking " apple in storage. This basis has the advantage that a simple calculation will transform a series of data into any other form, e.g. per unit of fresh weight, dry weight, water, etc. ; or of one nitrógen fraction as a proportion of total nitrogen.

During the first few hours of freezing to —20° C. the fruit loses an appreciable amount of water to the sides of the tins in which the samples are frozen. This loss amounts to 2-3 per cent. of the pre-freezing weight of the fruit. In the cases where the fruit is peeled there is also a small loss of about the same magnitude during peeling. Assuming that the latter loss consists of material uniform with the bulk of the tissue, use of the " per apple " basis for all calculations provides a simple means of correcting for both these losses. The number of apples comprising a sample as frozen is known so that any subsequent weight of the samples (pre-peeling weight ; weight of peel ; weight of pulp, etc.) is still represented by this number of fruits. The number of fruits represented by an aliquot portion of the tissue taken for analysis can be readily calculated. Loss of weight during storage experiments is also automatically corrected by this procedure.

(ii) " *Initial* " *respiration.*

With stored apples it is not difficult to obtain results for rate of respiration and for chemical composition which may be regarded, without risk of serious error, as comparable in time. The fruit will be at a constant temperature and changes, even in rate of respiration, will be negligible in the short time taken to " fix " the tissue by freezing.

When attempts are made to obtain similar data for fruit developing on the tree, complications ensue. Several additional factors become involved of which the most important are:

(*a*) The effect of detaching the fruit from the tree (i.e. removing its source of nutrients) on the rate of respiration and on chemical composition;
(*b*) Changes occurring within the fruit during removal from orchard to laboratory;
(*c*) Changes occurring while the fruit attains the temperature at which the rate of respiration is to be determined, and changes in chemical composition during cooling to —20° C. to await analysis.

With regard to factor (*a*), little is known concerning the early stages of development although experiments have shown that, in all probability, no great changes in chemical composition occur as a direct and immediate result of picking. Experience has also shown that from about 80-90 days from petal fall onwards all three factors are of no great importance. Prior to this stage, however, and of increasing importance as the fruit is less and less developed, the rapid fall in rate of respiration following picking makes comparative studies of rate of respiration and chemical composition extremely difficult. For example, in an apple gathered 18 days from petal fall, the rate of respiration may have fallen in the period 6-15 hours after picking from 1,420 ml. CO_2 per 10 kg. per hour to 740 ml. CO_2 per 10 kg. per hour (at 12° C.). Over the same period the fall in rate for an apple gathered 40 days from petal fall may be only from 300 to 285 ml. CO_2 per 10 kg. per hour, although in this case the subsequent fall may be more rapid.

It has long been known that the rate of respiration of young apples falls rapidly immediately after picking, but the speed and magnitude of this fall were not known accurately until the 1945 experiments had been carried out. These experiments were designed to elucidate the changes in respiration-rate and chemical composition of apples up to about 60 days from petal fall *within the first few hours of detachment from the tree.* The work will be reported in detail elsewhere, but the respiration-data obtained have been used in an attempt to calculate the " true " initial rate of respiration of the immature fruit of the 1936, 1938 and 1939 experiments.

In these latter experiments, factors (*b*) and (*c*) mentioned above were spread over periods up to 24 hours from picking and subsequent measurements of respiration rate were seldom made more frequently than over 12-hour periods. Consequently, it was upwards of 35-40 hours before the drift of respiration-rate was known accurately. This prompted the use, as an expedient, of the value of the rate of respiration at 40 hours from gathering as a measure of " initial " respiration rate. It is this figure which is recorded in the tables as the " observed 40 hour " rate of respiration.

By a series of fortunate coincidences, on the days when the fruit was gathered in the 1945 experiments, the temperature of the orchard (and of the fruit) was never more than one or two degrees above or below 12° C. This fact, combined with rapid picking, enabled the rate of respiration to be measured accurately 4 to 5 hours after the detachment of the fruit from the tree. With subsequent measurements taken every 6-8 hours, a curve representing the drift in rate from 4-5 hours to upwards of 90 hours was obtained which, on interpolation to zero time, gave a value for the rate of respiration which may reasonably be taken as that of the rate of respiration (at 12° C.) obtaining at the moment the fruit was picked. For brevity, the terms " IR " and " 40 hour R " will be used to describe the " true " initial rate of respiration (the value of respiration rate at zero time interpolated from the curves of respiration rate in the 1945 experiment or calculated therefrom) and the rate of respiration at 40 hours from gathering, respectively. Unless otherwise stated, the latter term will represent an observed rate of respiration.

The results obtained in the 1945 experiment confirmed and emphasized the divergence between the 40-hour value of R and the probable true initial value of R (IR), at least up to 50 days from petal fall. It thus became obvious that the 40-hour value was of little use as a measure of initial rate of respiration in ontogenic studies, at least during the early stages of development. The 40-hour value of R does, however, represent a definite point in the respiration-curve of the detached fruit and it appeared feasible that if, in the 1945 experiment a simple relationship could be found between " IR " and " 40 hour R " which was valid throughout the initial stages of development, the " IR " values for the 1936, 1938 and 1939 experiments could be calculated from the known " 40 hour R " values. Two facts must, however, be reiterated here. In the first place such an IR value would take no account of the direct effect of detachment from the tree on the rate of respiration of the fruit ; in the second place the above argument presupposes that the apples used in 1945, although from different trees, were sufficiently similar for a relationship found within the one set of fruit to be applicable to the other. There are not sufficient data at present available to assess the magnitude of the first factor which has therefore been ignored in subsequent calculations. The apples used in the two series of experiments, although from different orchards, were from trees on the same rootstock. This is the only justification for the second assumption. It is not proposed to discuss the validity of the method finally adopted to define a relationship between " IR " and " 40 hour R ". A strict theoretical justification of the method appears improbable and, for the present at least, its use can only be regarded as a convenience.

THE RELATIONSHIP DEVELOPED BETWEEN " IR " AND " 40 HOUR R ".

If the log of the interpolated value of R (IR) for the 1945 experiment and the log of 40 hour R in the same experiment are plotted against days from petal fall, the graphs obtained, while intersecting at about 70 days and exhibiting the rudiments of straight lines, are not sufficiently close to linearity towards the point of intersection to provide a basis for the development of a simple relationship between " IR " and " 40 hour R ". Furthermore, if the logs of the " 40 hour R " values for the 1936, 1938 and 1939 experiments, which themselves all exhibit broadly the same drift as the " 40 hour R " values for 1945, are plotted on the same graph as the two sets of data for 1945, the three lines obtained have no common point of intersection. There is obviously no simple relationship between these three lines.

A minimum rate of between 35 and 50 ml. CO_2 per 10 Kg. per hour was found for the respiration of pre-climacteric Bramley's Seedling apples (at 12° C.) after detachment from the tree. Thus the lowest value of R of a normal detached Bramley's Seedling apple is about 35 ml. CO_2 per 10 Kg. per hour. If it is assumed that this is the " basal " respiration of the fruit, the value of IR (as calculated below) of pre-climacteric fruit is 51 ml. CO_2 per 10 Kg. per hour. This agrees reasonably well with the values of 45-50 obtained for R during the first 40-50 hours after gathering for all pre-climacteric fruit of the 1936, 1938 and 1939 experiments at the stage when R changes very little after gathering (August onwards).

When log (IR—35) and log (40 hr. R—35) for the 1945 data and log (40 hr. R—35) for the 1936, 1938 and 1939 data were plotted against days from petal fall, a cursory glance at the resulting graph showed that the three sets of data fall on an approximately straight line and that the three lines intersect at between 80 and 85 days from petal fall. This procedure was, therefore, adopted as a working convention and the lines of best fit for each set of data were obtained by the method of least squares. These lines, together with the points corresponding to the observed values of (R—35), are shown in Fig. 1. Line A represents log (IR—35) for 1945, Line B represents log (40 hr. R—35) for 1945 and Line C represents log (40 hr. R—35) for all the 1936, 1938 and 1939 data up to 77 days from petal fall. These lines intersect at 83 (82·7) days from petal fall so that the " origin " of the graph is at 83 along the x-axis and 1·21 on the y-axis.

A line DO was next constructed from the relation $DX=\frac{CX \times AX}{BX}$ and this line was taken to represent the values of IR for the 1936, 1938 and 1939 experiments.

The equation of this line is of the form $y=x \tan. \alpha$, where α is the angle subtended by DO and the x-axis. For use in calculating the values of IR and the various picking dates of these experiments it was necessary to modify this equation to harmonize with the origin at point O and the scale on which the graph is drawn.

The final form of the equation was

$$y=[(83-x) \times \tan. 43\cdot6^\circ \times 0\cdot025]+1\cdot21$$

where y represents log (IR—35), x represents days from petal fall, $\alpha=43\cdot6^\circ$ and 0·025 is the factor necessary to harmonize the scales of the x and y axes.

From this equation the values in the following table were calculated for the 1936, 1938 and 1939 experiments.

TABLE I.

Days from Petal Fall (x)	log (IR—35) (y)	IR—35 ml. CO_2/10 kg. /hr.	IR ml. CO_2/10 kg. /hr.	IR mg. CO_2/apple /100 hrs.
1936 21	2·68	482	517	34·6
33	2·40	249	284	73·0
46	2·09	123	158	90·0
56	1·85	71	106	81·2
77	1·35	23	58	65·1
1938 34	2·37	236	271	63·0
62	1·71	51	86	79·5
1939 24	2·61	407	442	96·5
69	2·02	104	139	81·5
66	1·61	41	76	81·9

STATISTICAL TREATMENT.

As far as possible replicate samples were taken from time to time during the course of the experiments and the results for all chemical analyses were subjected to statistical treatment.

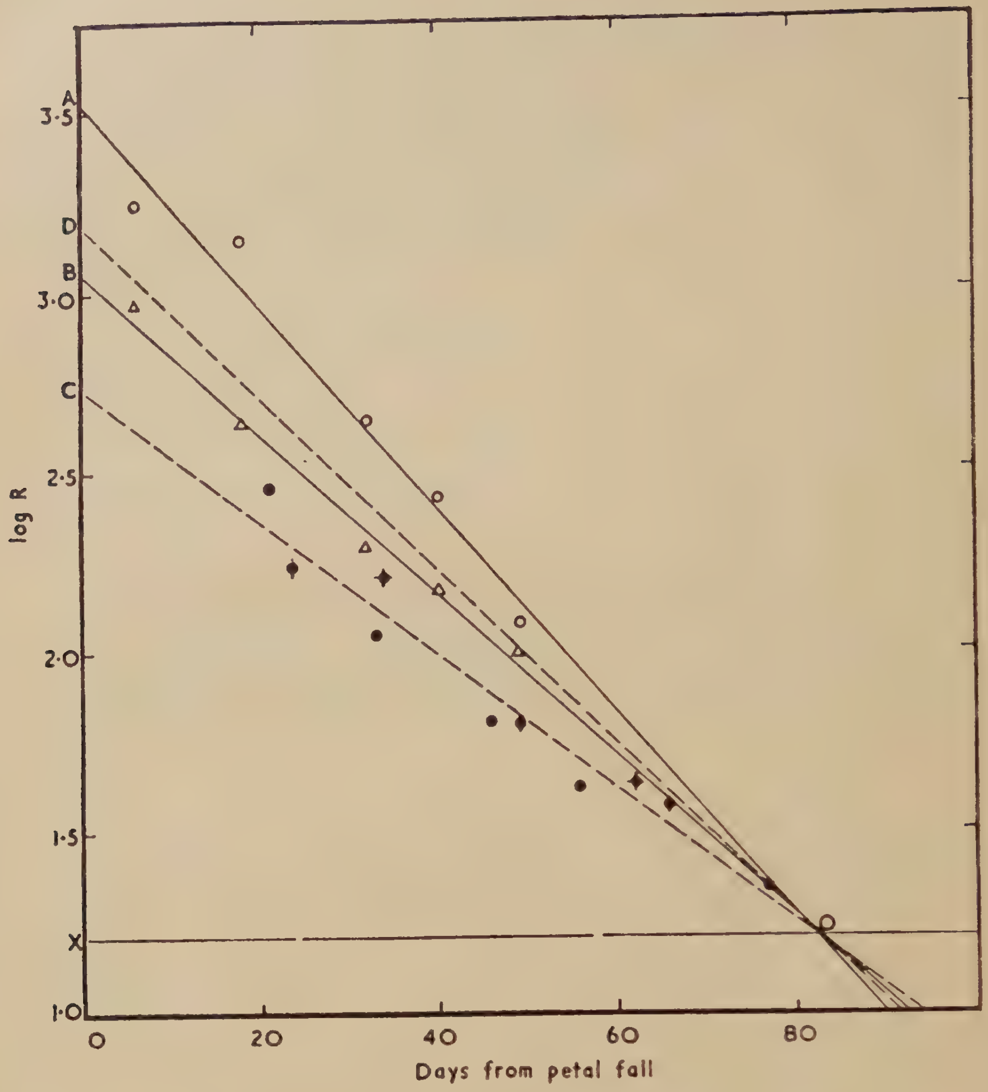

FIG. 1.

The relationship between log (IR − 35) and log (40 hour R − 35) and time for the 1945 experiment and the 1936, 38 and 39 experiments.

○ (IR − 35) } 1945 continuous lines calculated from the values represented by the respective points.
Δ (40 hour R − 35) }

● 1936
✦ 1938 } (40 hour R − 35); discontinuous line calculated from all 3 years' results.
♦ 1939

Discontinuous line without points marked—(IR − 35) for 1936, 38, 39.

EXPERIMENT VIII.

Four gatherings of Bramley's Seedling apples stored at 10° C. in air.
Source: Speedgate Farm, Farningham.

(a) *1st pick.*

Gathering date 19.6.39. *Number of apples per sample 100.* *Average weight 9·2 gm.*

Days in store.	Relative fresh weight gm.	Gm. per 100 gm. fresh weight.										Rots.	Respiration	
		Glucose.	Fructose.	Sucrose.	Starch after extraction with Kox.	Titratable acidity as malic.	Alcohol insoluble residue.	Alcohol soluble residue.	C content of alcohol insoluble residue by combustion.	C. content of alcohol soluble residue by combustion.	C. content of cleared solution by combustion.		Total CO_2 gm. per 100 gm.	Rate CO_2 ccs. per 10 K.H.
		Reducing sugar.												
0	98·80	1·30		0·23	0·25	—	—	—	—	—	—	—	—	—
0	98·24	1·54		0·27	—	—	—	—	—	—	—	—	—	—
0	98·65	1·42		0·26	—	—	—	—	—	—	—	—	—	—
0	98·80	1·51		0·26	—	—	—	—	—	—	—	—	—	—
0	98·52	1·44		0·24	—	—	—	—	—	—	—	—	—	—
0	98·65	1·52		0·25	—	—	—	—	—	—	—	—	—	—
0	98·87	1·38		0·28	—	—	—	—	—	—	—	—	—	—
Storage temperature 10°C.														
3	96·38	1·57		0·26	—	—	—	—	—	—	—	—	0·31	202
6	94·16	1·46		0·28	—	—	—	—	—	—	—	—	0·61	206
9	92·54	1·59		0·22	—	—	—	—	—	—	—	—	0·89	180
12	91·14	1·62		0·15	—	—	—	—	—	—	—	—	1·13	154
15	90·20	1·65		0·13	—	—	—	—	—	—	—	—	1·33	126
20	90·86	1·57		0·09	—	—	—	—	—	—	—	—	1·60	106
25	86·68	1·67		0·05	0·05	—	—	—	—	—	—	—	1·84	96
30	86·52	1·54		0·07	—	—	—	—	—	—	—	—	2·07	96
45	82·95	1·56		0·13	—	—	—	—	—	—	—	1 rot removed and 2 rots cut out.	2·71	80
60	82·70	1·43		0·14	—	—	—	—	—	—	—	1 rot removed.	3·25	70
75	80·08	1·44		0·13	—	—	—	—	—	—	—	—	—	—
100	77·00	1·36		0·15	—	—	—	—	—	—	—	3 rots removed.	—	—
115	75·73	1·22		0·19	0·05	—	—	—	—	—	—	—	—	—
130	71·34	1·17		0·25	—	—	—	—	—	—	—	2 rots removed.	—	—
Standard deviation of 7 initial samples expressed as percentage of each constituent		6·0		6·8	—	—	—	—	—	—	—	—	—	—

(b) *2nd pick.*

Gathering date 24.7.39. *Number of apples per sample 50.* *Average weight 52·0 gm.*

0	99·50	0·80	2·06	0·98	1·17	1·95	4·70	7·97	2·07	3·15	1·87	—	—	—
0	99·40	0·90	2·00	0·99	1·13	1·94	4·65	7·87	1·99	3·15	1·98	—	—	—
0	99·47	0·84	1·98	0·94	1·13	1·92	4·64	7·81	1·98	3·15	1·84	—	—	—
0	99·57	0·92	1·93	0·91	1·12	1·95	4·61	7·89	1·97	3·17	1·98	—	—	—
0	99·42	0·88	1·97	0·87	1·14	1·95	4·70	7·87	1·99	3·16	1·83	—	—	—
0	99·55	0·86	1·96	0·96	1·12	1·95	4·75	7·92	2·09	3·14	1·80	—	—	—
Storage temperature 10°C.														
3	98·64	1·11	2·04	0·93	0·89	1·93	4·39	8·18	1·88	3·23	2·20	—	0·11	70
6	97·96	1·08	2·17	1·05	0·55	1·97	4·02	8·48	1·77	3·41	2·05	—	0·20	64
9	97·76	1·24	2·31	0·98	0·38	1·92	3·77	8·60	1·60	3·48	2·18	—	0·29	56
12	97·26	1·27	2·34	0·92	0·21	1·97	3·63	8·68	1·60	3·53	2·19	—	0·36	50
15	96·94	1·33	2·44	0·93	0·15	1·96	3·52	8·84	1·54	3·52	2·16	—	0·42	44
25	96·43	1·49	2·72	0·72	0·15	1·89	3·34	9·07	1·44	3·63	2·32	—	0·64	42
25	96·26	1·43	2·64	0·70	0·15	1·90	3·39	9·09	1·45	3·74	2·32	—	0·64	42
30	95·42	1·43	2·74	0·52	0·16	1·85	3·34	8·90	1·41	3·53	2·40	—	0·75	42
30	96·42	1·62	2·56	0·58	0·16	1·86	3·32	8·99	1·45	3·53	2·21	—	0·75	42
30	95·82	1·53	2·66	0·51	0·15	1·83	3·24	8·96	1·40	3·52	2·48	—	0·75	42
40	95·16	1·48	2·79	0·49	0·14	1·82	3·35	8·85	1·45	3·68	2·19	—	0·96	42
50	94·17	1·55	2·87	0·44	0·15	1·72	3·24	8·70	1·42	3·51	2·47	—	1·16	40
62	95·06	1·59	2·75	0·27	0·15	1·65	3·22	8·54	1·40	3·55	2·46	—	1·38	40
62	94·58	1·60	2·82	0·33	0·12	1·61	3·16	8·56	1·36	3·59	2·30	—	1·38	40
62	94·08	1·53	2·84	0·23	0·12	1·63	3·20	8·56	1·40	3·41	2·37	—	1·38	40
70	94·46	1·57	2·92	0·26	0·11	1·59	3·21	8·55	1·36	3·40	2·27	—	1·52	38
70	93·45	1·43	2·82	0·30	0·12	1·53	3·16	8·48	1·42	3·49	2·39	—	1·52	38
70	93·60	1·60	2·83	0·28	0·15	1·55	3·18	8·52	1·42	3·43	2·38	—	1·52	38
80	93·40	1·46	2·77	0·31	0·10	1·53	3·12	8·41	1·36	3·49	2·27	—	1·73	50
90	92·39	1·40	2·84	0·31	0·11	1·48	3·14	8·29	1·34	3·47	2·13	—	1·98	54
100	93·24	1·36	2·76	0·36	0·12	1·46	3·13	8·18	1·35	3·35	2·30	1 rot removed.	2·25	60
120	90·62	1·40	2·70	0·32	0·10	1·30	3·08	7·83	1·33	3·24	2·16	—	2·76	50
140	88·55	1·25	2·71	0·37	0·10	1·20	3·04	7·66	1·29	3·23	2·09	—	3·24	50
160	89·75	1·27	2·55	0·23	0·11	1·10	3·04	7·31	1·35	2·98	2·02	1 rot removed.	—	—
Standard deviation of 6 initial samples expressed as percentage of each constituent		5·0	2·2	4·8	1·7	0·7	1·1	0·7	2·6	0·3	4·2	—	—	—

(c) *3rd pick.*

Gathering date 14.8.39. *Number of apples per sample 30.* *Average weight 72·08 gm.*

0	99·76	3·49		1·38	1·39	—	—	—	—	—	—	—	—	—
0	99·76	3·72		1·06	—	—	—	—	—	—	—	—	—	—
0	99·72	3·49		1·43	—	—	—	—	—	—	—	—	—	—
0	99·80	3·34		1·27	—	—	—	—	—	—	—	—	—	—
0	99·82	3·61		1·38	—	—	—	—	—	—	—	—	—	—
Storage temperature 10°C.														
3	99·09	3·71		1·43	—	—	—	—	—	—	—	—	0·09	60
9	98·36	4·14		1·67	—	—	—	—	—	—	—	—	0·24	46
12	97·62	4·41		1·61	—	—	—	—	—	—	—	—	0·31	40
15	97·84	4·39		1·60	—	—	—	—	—	—	—	—	0·36	36
20	97·10	4·76		1·37	—	—	—	—	—	—	—	—	0·44	36
25	96·54	4·89		1·20	—	—	—	—	—	—	—	—	0·53	34
30	95·70	4·93		0·97	—	—	—	—	—	—	—	—	0·61	35
41	95·86	5·27		0·84	—	—	—	—	—	—	—	—	0·79	34
50	94·35	5·30		0·77	0·12	—	—	—	—	—	—	—	0·98	50
60	94·76	5·58		0·60	—	—	—	—	—	—	—	—	1·23	50
70	94·07	5·58		0·47	—	—	—	—	—	—	—	—	1·47	51
80	93·75	5·28		0·49	—	—	—	—	—	—	—	—	1·73	54
90	92·47	5·25		0·36	0·16	—	—	—	—	—	—	—	—	—
111	90·38	5·38		0·22	—	—	—	—	—	—	—	—	—	—
130	85·42	5·04		0·34	—	—	—	—	—	—	—	2 rots removed.	—	—
Standard deviation of 5 initial samples expressed as percentage of each constituent		4·1		11·4	—	—	—	—	—	—	—	—	—	—

(d) *4th pick.*

Gathering date 2.10.39. *Number of apples per sample 25.* *Average weight 108·6 gm.*

0	99·72	5·23		2·38	0·62	—	—	—	—	—	—	—	—	—
0	99·60	5·05		2·54	—	—	—	—	—	—	—	—	—	—
0	99·82	5·03		2·42	—	—	—	—	—	—	—	—	—	—
0	99·80	5·19		2·39	—	—	—	—	—	—	—	—	—	—
Storage temperature 10°C.														
3	98·00	5·65		2·27	—	—	—	—	—	—	—	—	—	—
6	99·09	5·88		2·48	—	—	—	—	—	—	—	—	—	—
9	97·77	5·73		2·03	—	—	—	—	—	—	—	—	—	—
13	97·52	5·65		2·34	—	—	—	—	—	—	—	—	—	—
18	97·25	5·93		2·28	0·14	—	—	—	—	—	—	—	—	—
22	97·90	5·91		1·97	—	—	—	—	—	—	—	—	—	—
27	97·75	6·06		2·00	—	—	—	—	—	—	—	—	—	—
32	97·77	6·24		1·73	—	—	—	—	—	—	—	—	—	—
42	97·00	6·14		1·56	—	—	—	—	—	—	—	—	—	—
54	96·36	6·51		1·40	0·11	—	—	—	—	—	—	—	—	—
64	96·14	6·50		1·29	—	—	—	—	—	—	—	—	—	—
72	95·80	6·61		1·07	—	—	—	—	—	—	—	—	—	—
Standard deviation of 4 initial samples expressed as percentage of each constituent		1·9		3·0	—	—	—	—	—	—	—	—	—	—

TABLE III.

Bramley's Seedling apples (1936) during storage at 12° C.

Date harvested [illegible]	Days in storage	No. of replicate samples	Original fresh weight/apple gm.	% of Original fresh weight.	Pulp/peel.	per apple. Dry weight gm.	Total N mg.	Protein N mg.	Ammonia N mg.	Asparagine N mg.	Amino acid N [illegible]	[illegible]	[illegible]	[illegible]
Whole fruit														
11.6.	12	1	3·4	98·5	4·6	*0·265	6·9	4·1	0·07	1·7	0·[illegible]	[illegible]	*[illegible]	[illegible]
23.6.	9	1	13·4	99·3	5·9	*1·12	16·0	8·2	0·08	4·7	1·[illegible]	[illegible]	*[illegible]	[illegible]
,,	26	1	13·0	98·9	6·3	*1·07	16·2	7·8	0·10	5·6	0·[illegible]	[illegible]	*[illegible]	[illegible]
,,	101	1	12·9	80·7	6·3	*0·90	15·8	8·1	0·20	5·5	0·[illegible]	[illegible]	*[illegible]	[illegible]
6.7.	10	1	29·4	99·5	6·2	*2·37	23·5	12·6	0·09	6·8	1·[illegible]	[illegible]	*[illegible]	[illegible]
,,	25	1	28·4	99·0	6·1	*2·31	23·8	12·2	0·11	7·6	1·[illegible]	[illegible]	*[illegible]	[illegible]
,,	44	1	29·7	97·1	6·1	*2·37	25·6	12·0	0·19	9·3	1·[illegible]	[illegible]	*[illegible]	[illegible]
,,	57	1	29·6	90·6	6·0	*2·26	24·8	13·2	0·20	8·1	1·[illegible]	[illegible]	*[illegible]	[illegible]
,,	80	1	29·0	92·7	6·1	—	25·3	14·7	0·18	7·5	0·[illegible]	[illegible]	*[illegible]	[illegible]
16.7.	11	[illegible]	39·2	99·5	6·1	*3·4	29·9	16·3	0·04	8·8	2·[illegible]	[illegible]	*[illegible]	[illegible]
,,	25	[illegible]	39·3	98·9	6·1	*3·35	28·8	13·7	0·02	9·9	2·[illegible]	[illegible]	*[illegible]	[illegible]
,,	39	[illegible]	38·1	98·7	6·1	*3·31	30·5	14·2	0·19	10·8	2·[illegible]	[illegible]	*[illegible]	[illegible]
,,	55	[illegible]	38·8	98·0	6·0	*3·22	29·8	15·2	0·26	10·1	1·[illegible]	[illegible]	*[illegible]	[illegible]
,,	76	1	38·6	96·2	6·1	*3·09	28·6	16·8	0·30	8·1	1·[illegible]	[illegible]	*[illegible]	[illegible]
6.8.	11	[illegible]	57·3	99·7	7·4	*5·59	32·9	19·1	*0·02	*7·4	[illegible]	[illegible]	*[illegible]	[illegible]
,,	26	1	57·6	99·2	7·4	*5·55	30·2	20·1	*0·13	*9·4	[illegible]	[illegible]	*[illegible]	[illegible]
,,	41	1	56·0	97·8	7·4	*5·34	30·1	19·1	*0·19	*10·6	[illegible]	[illegible]	*[illegible]	[illegible]
,,	48	1	57·3	97·0	6·6	—	*27·2	*13·2	*0·15	*9·1	[illegible]	[illegible]	*[illegible]	[illegible]
23.8.	11	[illegible]	73·5	99·7	7·5	*7·64	39·0	22·5	0·06	10·2	[illegible]	[illegible]	*[illegible]	[illegible]
,,	26	1	73·8	98·7	7·7	*7·45	39·8	22·0	0·11	10·9	[illegible]	[illegible]	*[illegible]	[illegible]
,,	41	[illegible]	74·1	98·1	7·6	*7·23	39·9	24·9	0·18	9·6	[illegible]	[illegible]	*[illegible]	[illegible]
,,	57	[illegible]	74·5	98·3	7·6	*6·87	38·5	26·8	0·20	7·2	[illegible]	[illegible]	*[illegible]	[illegible]
31.8.	11	[illegible]	82·6	99·9	8·0	*8·76	40·3	24·5	*0·05	*8·9	[illegible]	[illegible]	*[illegible]	[illegible]
,,	31	[illegible]	84·2	98·8	7·9	*8·00	42·4	27·6	*0·15	*8·7	[illegible]	[illegible]	*[illegible]	[illegible]
8.9.	11	[illegible]	88·5	99·7	7·8	*9·20	42·9	25·0	*0·05	*10·1	[illegible]	[illegible]	*[illegible]	[illegible]
,,	13	[illegible]	88·5	99·6	8·0	*9·30	43·8	25·4	0·07	11·2	3·[illegible]	4·[illegible]	*[illegible]	[illegible]
,,	34	[illegible]	87·8	99·3	8·0	*8·90	42·1	27·7	*0·07	*8·6	*1·[illegible]	*2·[illegible]	*[illegible]	[illegible]
21.9.	11	[illegible]	102·2	99·7	8·3	*10·64	43·3	26·8	0·12	9·9	2·[illegible]	4·[illegible]	*[illegible]	[illegible]
,,	15	[illegible]	100·8	99·7	8·3	*10·53	43·4	27·7	0·12	9·3	2·[illegible]	3·[illegible]	*[illegible]	[illegible]
,,	18	[illegible]	100·1	99·5	8·3	*10·78	44·9	29·1	0·12	9·7	2·[illegible]	3·[illegible]	*[illegible]	[illegible]
,,	21	[illegible]	100·1	99·5	8·3	*10·63	42·6	29·5	0·12	7·7	1·[illegible]	3·[illegible]	*[illegible]	[illegible]
,,	26	[illegible]	100·3	99·2	8·3	*10·55	44·3	29·3	0·13	8·8	1·[illegible]	4·[illegible]	*[illegible]	[illegible]
5.10.	9	[illegible]	111·5	99·9	8·3	*12·56	48·0	32·7	0·14	8·1	2·[illegible]	4·[illegible]	*[illegible]	[illegible]
,,	14	[illegible]	116·7	99·7	8·4	*12·65	48·3	33·9	0·14	7·8	2·[illegible]	4·[illegible]	*[illegible]	[illegible]
,,	35	[illegible]	117·0	98·4	8·4	*12·06	48·4	35·0	0·21	7·1	2·[illegible]	3·[illegible]	*[illegible]	[illegible]
13.10.	11	[illegible]	117·5	99·7	8·4	*12·94	53·7	35·5	0·15	10·1	3·[illegible]	4·[illegible]	*[illegible]	[illegible]
,,	26	[illegible]	119·7	99·1	8·4	*12·65	49·9	36·1	0·19	7·0	2·[illegible]	3·[illegible]	*[illegible]	[illegible]
27.10.	15	1	124·0	99·5	8·5	*13·51	54·9	39·0	0·20	7·6	3·[illegible]	4·[illegible]	*[illegible]	[illegible]
†Standard deviation σ	—	—	—	—	—	0·147	0·70	0·66	0·031	0·43	0·[illegible]	0·[illegible]	[illegible]	[illegible]
Peel only.														
11.6.	12		See above—" whole fruit "			—	2·01	1·48	0·02	0·25	[illegible]	[illegible]	—	—
23.6.	9		..	..		—	4·02	3·03	0·02	0·50	[illegible]	[illegible]	—	—
,,	26		..	..		—	3·46	2·30	0·02	0·69	[illegible]	[illegible]	—	—
,,	101		..	..		—	3·08	2·05	0·03	0·65	[illegible]	[illegible]	—	—
6.7.	10		..	..		—	5·95	4·88	0·02	0·51	[illegible]	[illegible]	—	—
,,	25		..	..		—	5·30	4·02	0·04	0·65	[illegible]	[illegible]	—	—
,,	44		..	..		—	5·53	3·80	0·06	0·94	[illegible]	[illegible]	—	—
,,	57		..	..		—	5·51	4·07	0·05	0·85	[illegible]	[illegible]	—	—
,,	80		..	..		—	5·39	4·14	0·04	0·75	[illegible]	[illegible]	—	—
16.7.	11		..	..		—	7·30	5·90	0·01	0·55	[illegible]	[illegible]	—	—
,,	25		..	..		—	6·60	5·14	0·01	0·70	[illegible]	[illegible]	—	—
,,	39		..	..		—	6·10	4·58	0·06	0·73	[illegible]	[illegible]	—	—
,,	55		..	..		—	6·40	5·02	0·05	0·70	[illegible]	[illegible]	—	—
,,	76		..	..		—	5·87	4·85	0·06	0·58	[illegible]	[illegible]	—	—
6.8.	11		..	..		—	9·07	7·51	—	—	—	[illegible]	—	—
,,	26		..	..		—	8·28	6·63	—	—	—	[illegible]	—	—
,,	41		..	..		—	7·74	6·25	—	—	—	[illegible]	—	—
,,	48		..	..		—	—	—	—	—	—	[illegible]	—	—
23.8.	11		..	..		—	10·32	8·61	0·02	0·78	0·[illegible]	0·[illegible]	—	—
,,	26		..	..		—	10·09	8·33	0·03	0·89	0·[illegible]	0·[illegible]	—	—
,,	41		..	..		—	10·14	8·77	0·03	0·68	0·[illegible]	0·[illegible]	—	—
,,	[illegible]		—	—		—	[illegible]	[illegible]	[illegible]	[illegible]	0·[illegible]	0·[illegible]	—	—
31.8.	11		—	—		—	10·84	9·14	—	—	—	—	—	—
,,	31		—	—		—	11·06	9·73	—	—	—	—	—	—
8.9.	11		—	—		—	11·12	9·25	—	—	—	—	—	—
,,	13		—	—		—	11·16	9·29	0·02	0·85	0·4[illegible]	0·55	—	—
,,	34		—	—		—	11·04	9·70	—	—	—	[illegible]	—	—
21.9.	11		—	—		—	12·15	10·47	0·03	0·80	0·3[illegible]	[illegible]	—	—
,,	15		—	—		—	11·90	10·35	0·03	0·74	[illegible]	[illegible]	—	—
,,	18		—	—		—	12·35	10·77	0·03	0·75	[illegible]	[illegible]	—	—
,,	21		—	—		—	12·07	10·72	0·03	0·61	[illegible]	[illegible]	—	—
,,	26		—	—		—	12·11	10·81	0·03	0·55	[illegible]	[illegible]	—	—
5.10.	9		—	—		—	13·55	12·08	0·02	0·56	[illegible]	[illegible]	—	—
,,	14		—	—		—	13·21	11·07	0·03	0·38	[illegible]	[illegible]	—	—
,,	35		—	—		—	13·34	12·14	0·04	0·31	[illegible]	[illegible]	—	—
13.10.	11		—	—		—	14·70	13·26	0·02	0·44	[illegible]	[illegible]	—	—
,,	26		—	—		—	14·06	12·93	0·02	0·30	[illegible]	[illegible]·45	—	—
27.10.	15		—	—		—	15·27	13·96	0·02	0·38	[illegible]	[illegible]	—	—
†Standard deviation σ	—	—	—	—	—	—	0·29	0·25	0·014	0·075	[illegible]	[illegible]	—	—

* Pulp only (not whole fruit).

† σ is calculated from the replicate samples, initial as well as storage.

TABLE IV.

Bramley's Seedling apples and Early Victoria apples during storage at 12° C. (whole apple)

[illegible]	Days in storage	No. of [illegible]	Original fresh weight apple, gm	% of original fresh weight	[illegible] gm	[illegible] mg	[illegible] mg	Ammonia N mg	Asparagine N mg	Amino acid N mg	[illegible] mg	[illegible] mg	[illegible]	[illegible]	[illegible]	[illegible]
Bramley's Seedling, [illegible]																
[illegible]	[illegible]	1	[illegible]	[illegible]	1·356	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·1	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	[illegible]	[illegible]	1·247	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·3	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	[illegible]	[illegible]	1·229	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·1	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	2	[illegible]	[illegible]	1·210	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·0	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·4	[illegible]	1·133	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·1	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	[illegible]	[illegible]	1·102	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·2	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·2	[illegible]	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·2	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	[illegible]	[illegible]	1·016	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·2	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·5	[illegible]	0·987	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·1	—	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	48·6	[illegible]	6·139	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·6	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	46·9	[illegible]	5·538	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2·3	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	48·4	[illegible]	5·728	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2·0	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	47·8	[illegible]	5·549	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2·1	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	46·6	[illegible]	5·285	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2·0	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	46·9	[illegible]	5·221	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2·2	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	46·0	[illegible]	4·922	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1·7	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	47·0	[illegible]	4·769	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2·3	—	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	[illegible]	[illegible]	15·82	[illegible]	[illegible]	0·10	[illegible]	[illegible]	2·9	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	107·4	[illegible]	15·64	[illegible]	[illegible]	0·13	[illegible]	[illegible]	3·2	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	108·7	[illegible]	15·56	[illegible]	[illegible]	0·11	[illegible]	[illegible]	2·0	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	108·1	[illegible]	15·06	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3·2	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	107·1	[illegible]	14·35	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2·7	—	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	145·0	[illegible]	21·01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3·5	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	143·8	[illegible]	21·21	[illegible]	[illegible]	[illegible]	5·9	[illegible]	3·0	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	142·7	[illegible]	20·62	[illegible]	[illegible]	[illegible]	5·9	4·7	2·7	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	144·2	[illegible]	20·23	[illegible]	[illegible]	[illegible]	5·9	4·7	2·9	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	142·3	[illegible]	19·90	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3·5	—	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	180·9	[illegible]	27·72	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	179·2	[illegible]	26·49	[illegible]	[illegible]	[illegible]	[illegible]	4·0	4·1	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	[illegible]	[illegible]	26·26	[illegible]	[illegible]	[illegible]	5·8	[illegible]	[illegible]	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	[illegible]	[illegible]	25·83	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4·1	—	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	199·0	[illegible]	29·43	[illegible]	[illegible]	[illegible]	6·4	4·5	[illegible]	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	198·3	[illegible]	29·74	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	198·2	[illegible]	28·24	[illegible]	[illegible]	[illegible]	[illegible]	4·7	[illegible]	—	[illegible]	[illegible]	[illegible]	—
* [illegible]		—	—		0·35	[illegible]	[illegible]	0·011	1·21	0·34	[illegible]					
Bramley's Seedling, [illegible]																
[illegible]	[illegible]	1	11·1	[illegible]	1·203	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·0	[illegible]	1·184	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·3	[illegible]	1·154	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	2	11·1	[illegible]	1·130	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·0	[illegible]	1·076	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·0	[illegible]	0·960	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·0	[illegible]	0·925	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	11·0	[illegible]	0·880	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	30·2	[illegible]	3·375	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	30·1	[illegible]	3·416	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	29·8	[illegible]	3·235	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	29·6	[illegible]	3·146	24·2	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	29·6	[illegible]	2·983	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	30·1	[illegible]	3·002	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	29·5	[illegible]	2·851	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	29·2	[illegible]	2·716	22·1	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	54·4	[illegible]	6·884	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	56·2	[illegible]	7·200	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	54·7	[illegible]	6·938	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	54·0	[illegible]	6·688	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	54·7	[illegible]	6·228	[illegible]	[illegible]	—	—	—	—	2740	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	54·8	[illegible]	6·227	30·9	[illegible]	—	—	—	—	2510	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	55·8	[illegible]	5·870	32·3	[illegible]	—	—	—	—	2205	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	95·6	[illegible]	12·61	[illegible]	[illegible]	—	—	—	—	4140	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	95·8	[illegible]	12·37	[illegible]	[illegible]	—	—	—	—	4275	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	98·5	[illegible]	12·53	[illegible]	[illegible]	—	—	—	—	5305	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	99·9	[illegible]	13·10	[illegible]	[illegible]	—	—	—	—	5075	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1	[illegible]	[illegible]	[illegible]	44·1	[illegible]	—	—	—	—	5031	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	6004	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	7245	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	47·2	[illegible]	—	—	—	—	7132	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	8170	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	9420	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	9635	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	9410	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	9420	[illegible]	[illegible]	[illegible]	[illegible]
* [illegible]					[illegible]	1·34	[illegible]	—	—	—	—	121 (4·0%)	[illegible]	[illegible]	—	—
Early Victoria																
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
* [illegible]					[illegible]	[illegible]	[illegible]	—	0·73	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	—

* [illegible]

TABLE V.

Bramley's Seedling apples (1938) during storage at 10° C. in air and in 10-15% CO_2.*

Days in store.	No. of replicate samples.	Treatment.	Original fresh weight per apple gm.	% of original fresh weight	Total N per apple mg.	Soluble N per apple mg.	Protein N (by diff.) per apple.	Respiration rate mg. per 100 hours per apple.
0	4		150·7	100	55·2	19·7	35·5	—
8	1	Air	144·4	100	47·6	15·2	32·4	114
8	1	CO_2	146·9	100	46·9	13·8	33·1	120
27	1	Air	146·5	99·7	47·9	14·5	33·4	86
27	1	CO_2	146·8	99·8	52·0	17·0	35·0	82
43	1	Air	151·0	99·4	55·4	15·4	40·0	168
43	1	CO_2	151·5	99·6	55·1	19·5	35·6	63
56	2	Air	148·7	99·3	51·6	10·4	41·2	169
56	2	CO_2	148·2	99·6	53·1	16·7	36·4	48
78	1	Air	151·3	98·9	54·9	13·5	41·6	158
78	1	CO_2	146·4	99·5	53·7	17·0	36·7	70
100	1	Air	151·6	99·0	54·6	11·1	43·5	128
100	1	CO_2	150·9	98·8	52·7	14·3	38·4	83
147	1	Air	145·8	98·5	49·1	11·4	37·7	118
147	1	CO_2	147·5	99·3	51·2	14·2	37·0	80
Standard deviation = σ	—	—	—	—	3·4	2·4	1·2	—

* These data refer to an experiment separate from the one the results of which are recorded in Table III. The fruit used was, however, from the same trees in both cases. The measurements of rate of respiration were carried out by Mr. H. J. Moore at the Low Temperature Research Station by a special gasometric technique (7).

Standard deviations were calculated from the following equations (6):

Variance$=\Sigma\delta^2$/degrees of freedom, δ being the deviation of replicates from their mean.

Standard deviation, $\sigma=\sqrt{\text{variance}}$.

The relevant standard deviations are shown at the foot of the tables.

In the case of carbohydrates, the amounts present "per apple" in a mature fruit may be several hundred times as great as that in very young fruits. The same error involved in a determination may be multiplied considerably in the former as compared with the latter owing to the greater dilution necessary in the actual determination of these constituents.

For sugars and starch therefore, the percentage standard deviation was calculated using the *percentage* deviations from the mean in each case for δ. These percentage standard deviations are shown in brackets in the Tables after the corresponding "normal" standard deviations.

ACKNOWLEDGMENT.

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THE DEGRADATION OF STARCH IN APPLES REMOVED FROM THE TREE AT DIFFERENT STAGES OF DEVELOPMENT

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The present paper is based mainly on data obtained in 1934 with Bramley's Seedling apples. Data on Worcester Pearmain apples obtained in 1935, and also on Bramley's Seedling apples in 1937 and 1939 are dealt with. These data and the methods of analysis used have already been published in full in this Journal (Griffiths, Potter and Hulme, 1950) and are therefore not included here.

The fruit for the 1934 experiment was gathered on 10th July, when the average weight of an apple was 30 gm. A large number of uniform samples of 40 apples were stored at each of the following temperatures, 1°, 3°, 10°, 15° and 20° C. Eight samples were analysed as replicate "initial" samples.

Starch begins to appear in the apple about the middle of June, two or three weeks after petal fall, the amount present in Bramley's Seedling apples reaching a maximum of 1 to 2 per cent. late in August and, by the middle or end of October, decreasing again to zero (Archbold, 1932). That starch disappears very quickly from apples when they are gathered at any stage of maturity is well known and the present study is an attempt to trace the course of this process.

When the starch in apples is determined by the takadiastase method (Griffiths and Potter, 1945) it is always found that after a rapid disappearance of starch in the early days of storage a residuum remains throughout the storage life of the apple. This residuum, which we shall call Fraction (C), has the following characteristics: It is extractable from the alcohol-insoluble fraction by treatment with 1 per cent. potassium oxalate solution (Kidd, West, Griffiths and Potter, 1940; Griffiths and Potter, 1945). It is not hydrolysed by β-amylase (Hanes, 1936). It yields reducing substances on hydrolysis with takadiastase. Finally, it is probably not extractable from the alcohol-insoluble residue by hot water (Hanes, 1936).

Hanes (1936) tested his β-amylase method for the estimation of starch on aliquots of the actual material stored at 15° C. in the 1934 experiment, and obtained a starch content of approximately 0·60 per cent. at half-day's storage and a curve for starch disappearance which fell practically to zero (0·01 per cent.) in the first ten days and showed only a very small residuum (0·007 per cent.) after 190 days. Unfortunately no observations were made on the eight initial samples.

In comparing his results with those obtained by us with the takadiastase method, the difference values plotted against time give a curve falling during the first ten days from a half-day value of about 0·35 to 0·15 per cent. after ten days, and then remaining approximately constant over the whole period of storage.

Hanes (loc. cit.) also found that in samples of apples taken from storage at 15° C. after half a day, about two-thirds of the fraction hydrolysed by takadiastase, but not by β-amylase, was extractable with hot water whilst one-third was not, this proportion being approximately that between the initial and the residual amounts as shown by the difference-curve mentioned above. There is thus evidence of a second fraction which we shall call Fraction (B) and which disappears in the apples after gathering, in step with the disappearance of starch. This fraction would appear from Hanes' observations to have the properties (i), of not being hydrolysable by β-amylase and (ii), of probably being extractable with hot water.

Two of the present authors (Griffiths and Potter, 1945) have shown that there is a fraction of the alcohol-insoluble residue from both Bramley's Seedling and Worcester Pearmain apples which is extractable by cold water or by potassium oxalate in the cold and which gives a purplish-blue colour with iodine. This fraction is present when the fruit is gathered but disappears from the fruit at the same time as the starch.

The indications are at present that the purple-staining dextrin constitutes Fraction (B) and that this dextrin is resistant to hydrolysis by β-amylase. It thus recalls in its properties the α amylodextrin of Baker (1902), which is a resistant dextrin formed in about 40 per cent. yield by the completed action of β-amylase on starch. Hanes has pointed out to us (personal communication) that a somewhat similar resistant fragment of the amylopectin molecule is probably formed by the action of amylase-phosphorylase since, like β-amylase, this enzyme appears to attack the free chain ends and to be stopped in the vicinity of branching points. If therefore it were possible positively to identify the purple-staining dextrin either as α amylodextrin or as the dextrin left by amylase-phosphorylase action, it would constitute valuable evidence as to the enzymic mechanism whereby starch is degraded in the apple fruit. Whether an amylase or a phosphorylase type of mechansim is involved, it is clear that Fraction (B) gives rise to reducing sugars in the plant.

The total fraction hydrolysable by takadiastase in the apples at the time of gathering, in the 1934 experiment, may thus be considered to be made up approximately as follows: Fraction (A)=starch, 0·60 per cent.; Fraction (B)=purple-staining dextrin, 0·15 to 0·2 per cent.; and Fraction (C)=0·10 to 0·15 per cent. Figure 1 sets out diagrammatically the behaviour of these three fractions after gathering.

The present data suggest that Fraction (C) may diminish slightly in quantity during prolonged storage and that it is slightly larger at low temperatures than at high ones. That this fraction exists at the time of gathering, as indicated in the diagram, is conjectural only.

Fractions (A) and (B) are only distinguishable in the Bramley's Seedling apples stored at 15° C. studied by Hanes and in the Worcester Pearmain apples studied by Griffiths and Potter. They are indistinguishable in the remaining data of the Bramley's Seedling apples and are so treated in what follows except when otherwise stated.

Preliminary inspection of the 1934 data in quantity/time plottings suggests that the rate of loss of Fractions (A)+(B) increases in the first day or two and then steadily falls. Now it is known that in Bramley's Seedling apples, at the stage of development of those used in 1934, Fraction (A) is being formed in the fruit on the tree at the rate of 0·02 gm. per day per fruit ; or 0·07 gm. per day per 100 gm. fresh weight (Archbold, 1932 ; Widdowson, 1932). That the process of synthesis or condensation of starch which is proceeding on the tree should cease instantaneously when the fruit is picked is unlikely.

The general form of the quantity/time curve for starch loss can be reasonably interpreted on the following assumptions:

(1) That the process of starch synthesis which is in progress on the tree continues for a short time, one or two days after gathering, and then rapidly falls to zero.

(2) That during this period, and subsequently, the process of degradation of starch is proceeding at a rate proportional either to the amount of starch present or to the surface area of the starch grain.

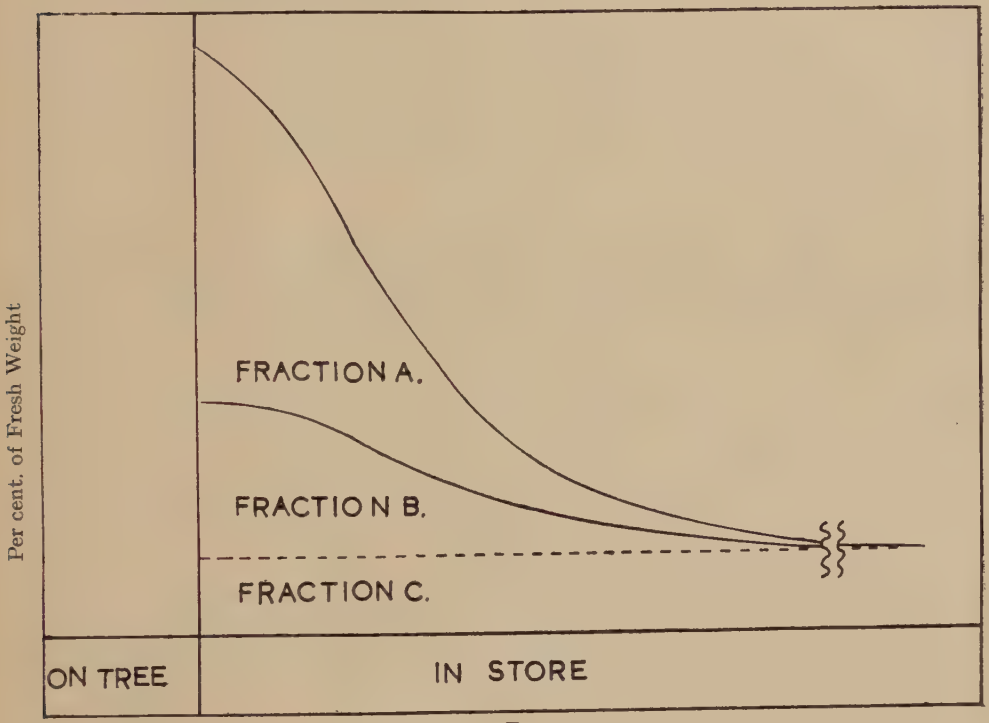

FIG. 1.

Diagram showing the fractions hydrolysed by takadiastase (see text).

(3) That degradation and synthesis of starch may be independent and simultaneously occurring processes.

From the above it would follow that starch degradation may be continuously in progress in the growing apple on the tree all the time that starch is present, that is throughout the main period of growth by cell enlargement from mid-June until the end of October. If so, this has important consequences as will appear in a later paper where the products of starch degradation after gathering will be examined and the results compared with the history of sugar accumulation in the fruit during growth.

Among the conditions brought about by gathering, which appear to lead to the cessation of starch synthesis, the most likely would appear to be the cutting of the translocation stream of carbohydrate from the tree. It may be assumed that precursors of starch are present in the translocation stream in the apple at the moment of gathering, in quantity sufficient to yield about 0·1 gm. per 100 gm. of tissues, this being approximately the extent of synthesis assumed to occur after gathering, which would adequately account for the shape of the quantity/time curve of starch loss during the first day or two.

To determine whether the above assumptions can be substantiated, the data obtained in 1934 with Bramley's Seedling apples have been more critically examined.

The procedure adopted was to plot the logarithms and the cube roots of the weight of starch in grams per 100 gm. original fresh weight against time in days, using as the weight of starch the observed value minus the Fraction (C) which persists throughout storage life.

The constants of the straight lines of best fit were then calculated for the Equations I and II, assuming errors to be in the starch variable and not in the time variable.

I. $\log W = b - at$

II. $\sqrt[3]{W} = c - mt$

where b, a, c and m=constants and t=time in days.

These calculations were made in two ways: Procedure (a), for the whole set of points, including the eight initial points at zero time; and Procedure (b), for the points from the first day onwards, omitting the initials and those during the first half-day.

EXAMINATION OF THE LINEAR RELATIONSHIP OBTAINED FROM THE DATA OF 1934 (BRAMLEY'S SEEDLING APPLES).

The linear relationship postulated in Equation I should hold if the rate of degradation were proportional to the mass of starch present and that postulated in Equation II should obtain if it were proportional to the surface area of the starch grains, which are approximately spherical.

When Ia and IIa were compared it became obvious that a linear relationship was not shown by the observations in either case. With one exception, all the observations at each temperature during the first four days fell above the line, while the majority of the initial eight observations and their mean fell in all cases below the line.

A comparison of Ib and IIb was then made and it was found that the scatter of the observations above and below the line was random in the case of IIb (Fig. 2), but in that of Ib, the early and late points were mainly below the line while the intermediate points were mainly above. In both cases all the lines cut the zero time axis well above the values of the eight initial samples. An analysis of variance showed an insignificant departure from a linear relationship in the case of IIb, but a significant one in the case of Ib.

The conclusion may therefore be drawn that after a short initial period during which starch synthesis acts as a delaying factor, the observations conform to the hypothesis that degradation of starch alone is proceeding and that the rate of degradation is proportional to the surface area of the starch grains.

EFFECT OF TEMPERATURE ON THE LINEAR RELATIONSHIP.

The divergence from a linear relationship between the cube root of the weight of starch and time during the first few days is greater at 20° C. and 15° C., than at 3° C. and 1° C. This may be seen by comparing the values in Table I below for the constants " m " and " c " in IIa and IIb where " m " defines the slope and " c " the point of origin on the zero time axis.

TABLE I.

Temperature (°C.).	Values of constant " m ".		Values of constant " c ".		$\sqrt[3]{\text{mean}}$ of 8 initial samples.
	IIa.	IIb.	IIa.	IIb.	
20	·088	·104	·906	·948	0·90
15	·072	·090	·912	·984	
10	·060	·068	·899	·927	
3	·044	·046	·902	·920	
1	·036	·038	·902	·922	

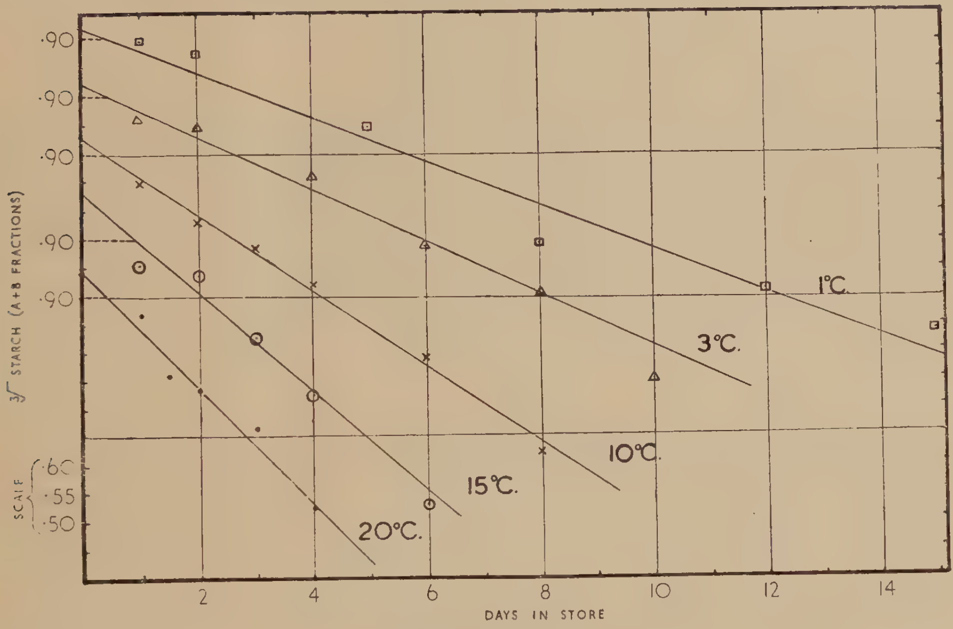

FIG. 2.
(For explanation, see text.)

It is seen that IIb has a steeper slope at 20° C. and 15° C., than IIa, but that this is not the case at 3° C. and 1° C. The inference is tentatively drawn that synthesis or condensation of starch from carbohydrate is more retarded at low temperatures than is the process of degradation. The values of the constant " m " in IIb define the rate of starch degradation per unit surface area of the starch granule at any given value for surface area, and the relative rates for the temperatures studied are :—

Temperature ..	1° C.	3° C.	10° C.	15° C.	20° C.
Relative rate ..	1	1·21	1·79	2·36	2·74

The temperature co-efficient of the degradation process is clearly relatively small.

Fraction (B) now has to be dealt with. This fraction appears to behave like starch in that it disappears quickly from the fruit after gathering at the same time as Fraction (A). Hanes' (loc. cit.) observations on the samples of Bramley's Seedling apples stored at 15° C. in the 1934 experiment suggest that after gathering, the loss of this Fraction (B) begins slowly and then accelerates after the first two days as in the case of the combined Fractions (A) and (B) which has already been discussed. Owing to the limitation of Hanes' observations to one temperature, and to the omission of the eight initial samples, there is no clear indication in this experiment that the divergence from the cube-root relationship during the first two days in the case of the combined Fractions (A) and (B) may not be due entirely to the inclusion of Fraction (B). That both Fractions (A) and (B) are, however, affected by the factor which delays the degradation of starch is shown by examination of the data obtained from experiments of a similar type which were carried out with Worcester Pearmain apples during 1935, and with Bramley's Seedling apples in 1937 and in 1939.

STARCH FRACTIONS IN WORCESTER PEARMAIN APPLES.

In the experiment with Worcester Pearmain apples there were four gatherings between July and September and four initial samples in each gathering. The fruit was stored at 10° C. and 1° C. in samples containing 30 apples each. The starch was determined by hydrolysis with takadiastase with and without previous extraction of the alcohol-insoluble residue with 1 per cent. potassium oxalate solution, and by the β-amylase method of Hanes in the case of the third gathering only. The data have been published by Griffiths and Potter (1945).

These results have now been examined by the formal Procedure b described above, and the constants " m " and " c " for the lines of best fit for Equation II are shown in Table II.

TABLE II.

Gathering.	Temperature (°C.).	Starch (Fractions A + B).			Starch (Fraction A).		
		" m "	" c "	$\sqrt[3]{\text{mean}}$ of 4 initials.	" m "	" c "	$\sqrt[3]{\text{mean}}$ of 4 initials.
18/7/35 ..	10	·044	1·362	1·247	·048	1·371	1·234
29/7/35 ..	10	·056	1·448	1·280	·051	1·400	1·274
15/8/35 ..	1	·020	1·326	1·320	·020 (·020)*	1·282 (1·282)*	1·288
,, ..	10	·049	1·465	1·322	·048 (·051)*	1·397 (1·426)*	1·288
2/9/35 ..	10	·047	1·276	1·193	·049	1·246	1·142

* The values in brackets are for results obtained by estimation with β-amylase.

The relationship postulated in Equation II holds after the first few days, the results in the case of the third and fourth gatherings being highly significant in this respect, and those of the first and second less so. In all cases of storage at 10° C. the cube roots of the four initial values at zero time fall significantly below the straight line of best fit through the cube roots of the amounts plotted against time (Procedure b). This is shown to be equally true for starch alone (Fraction (A)), and for starch + purple-staining dextrin (Fraction (A) + (B)). The factor which delays the degradation therefore affects both the (A) and the (B) fractions. When however, a sharp lowering of temperature is experienced immediately after the fruit is gathered, as in the case of the samples of the third gathering stored at 1° C., this delay does not occur. The observations on Worcester Pearmain apples thus support the tentative inference drawn from the data for the Bramley's Seedling apples in 1934, namely that the synthesis or condensation of starch from carbohydrates in the translocation paths is more markedly retarded at low temperatures than is the process of starch degradation.

That the rate of starch degradation is slower in Worcester Pearmain than in Bramley's Seedling apples is suggested by the values for the constant " m " (Equation II) which are given in the above tables. This rate is not greatly affected, if at all, by the stage of development of the fruit on the tree during the period studied.

EXAMINATION OF DATA OF THE 1937 AND 1939 EXPERIMENTS WITH BRAMLEY'S SEEDLING APPLES.

Further experiments with Bramley's Seedling apples were carried out in 1937 and 1939. In 1937, the apples were gathered on 24th August at an average weight of 135·7 gm. They were stored at temperatures of 15°, 10°, 5° and 1° C. In 1939, they were gathered on 24th July at an average weight of 51·6 gm., and stored at 10° C. In both cases, starch (Fraction (A)) was determined by hydrolysis of the alcohol-insoluble residue with takadiastase after extraction of the residue with 1 per cent. potassium oxalate solution.

These data have also been examined by the formal Procedure b, described above, and the constants " m " and " c " for the lines of best fit for Equation II are shown in Table III.

TABLE III.

Gathering.	Temperature (°C.).	Constants.		$\sqrt[3]{\text{mean of 10}}$ initial samples.	Relative rate for starch degradation.
		" m "	" c "		
24/8/37 ..	15	·062	1·43	1·24	2·58
,,	10	·035	1·25	1·24	1·46
,,	5	·034	1·28	1·24	1·42
,,	1	·024	1·32	1·24	1·0
24/7/39 ..	10	·055	1·09	1·00*	—

* Six samples only.

These results confirm those of the 1934 Bramley's Seedling experiment and show that the effect on the starch (Fraction (A)) of the delaying factor is more pronounced at 15° C. than at 1° C.

SUMMARY.

1. The results of starch determinations made on Bramley's Seedling and Worcester Pearmain apples after gathering indicate the presence of three fractions hydrolysable by takadiastase, namely: (A) starch, (B) a purple-staining dextrin and (C) a residuum which persists throughout storage life.

2. Examination of the data suggests that the process of degradation of starch proceeds at a rate proportional to the surface area of the starch grain after a short initial delaying period during which synthesis of starch, which was in progress on the tree, continues at a diminishing rate for one or two days after gathering.

3. The delay in starch degradation during the first day or two of storage affects both the starch and the (B) fraction.

4. The synthesis or condensation of starch from carbohydrate is more retarded at low temperatures than is the process of starch degradation.

The work described in this paper was carried out as part of the research programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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REVIEWS

GLOUCESTERSHIRE, SOMERSET AND WILTSHIRE: A GEOGRAPHICAL ANALYSIS. University of Bristol Reconstruction Research Group. (Pp. 12 + 4 Plates. 3 Maps. Bristol, 1949. Price 8s. 6d. net.)

The Reconstruction Research Group of Bristol University was established in 1943 to analyse the factors, geographical, sociological and other, which have bearing on the problems of planning and future development of the three English counties included in the Bristol area. It was realized that a general geographical analysis was a prime essential but would take a considerable time, and it was decided to determine by analysis " those areas in which industry and its resulting settlement may be developed with maximum advantage and with a minimum disadvantage to agriculture, to scenic and other amenities and above all to the people already settled in the areas concerned ". To this end, specially drawn maps were prepared showing features of topography, land classification and special interest and these were then combined in a single composite map to demonstrate those parts of the region in which industrial development is either impossible or undesirable on physical, human or economic grounds. The maps showing topography and land classification and the composite map are here published on the scale of a quarter-inch to the mile.

The work in general follows a similar line to that of the West Midland Group in their survey of the five counties centred on Birmingham. The analysis of land classification has been previously published and is here summarized to explain the basis of the classification.

The publications of these two groups constitute the first attempt at serious scientific, and therefore objective, study of the factors which it is so necessary should be taken into account in the future industrial development of the country. All too often the planning of new industrial areas is undertaken without previous analysis of the existing over-all picture and it is left to hastily-conceived and often ill-informed bodies subsequently to dispute points which should never have escaped attention in the earliest stages. The groups are contributing a service of unique value and one which should be extended to cover the whole country.

R.H.S.

THE PLUMS OF ENGLAND. By H. V. Taylor. (London: Crosby Lockwood & Son, Ltd. 1949. Pp. 151 + Bibliography, pp. 30 and 32 coloured Plates. Price 30s.)

Part I of this book contains much useful information on cultural considerations and includes botanical notes of the various species of plum grown in this country.

The chapters on the botanical characters of plum species, on rootstocks and pollination bring together much useful information, previously published elsewhere, both for the student and the grower, and the account of breeders of plum varieties gives recognition to fruit breeders of the past and also to Messrs. Laxton of Bedford, Mr. G. T. Spinks and Mr. M. B. Crane who have produced new varieties of promise during recent years and are continuing in this work. The botanical notes would be more helpful to the fruit grower, however, if such formulae as $2n=48$ etc., were explained instead of appearing as mere statements. Little new ground is covered under the heading of "Botanical considerations and Classes of Plums" and the reviewer draws attention to the urgent necessity for systematic pomological work on plums. Actually much has been done, but not published, in this direction by Mr. J. M. S. Potter of the National Fruit Trials, who gave a paper at the R.H.S. Hall in 1936 entitled "A tentative classification of Plums based on their morphological characters".

In describing the technique of hand pollination in breeding new varieties, the importance of emasculating the flower of the female parent is stressed but care in preventing contamination of the stamens of the male parent is not mentioned. The variety Thames Cross is omitted in reference to Mr. G. T. Spinks' work at Long Ashton.

The information on blossom periods and pollination is well assembled and the tables showing length and relative period of blossoming of many varieties should be of help in laying out new plantations.

The brief survey of plum-growing areas and the varieties grown emphasizes the author's contention that the English plum-growing industry has been largely built up on culinary and processing varieties and that heavy cropping capacity rather than quality has been the criterion. The author shows, however, that sufficient is now known of pollination requirements, soils, nutrition, etc., to make the growing of quality plums an attractive proposition. Indeed this book will have justified its publication if it only focuses attention on dessert and other plums of quality which can be grown successfully in England. Diseases and pests are described in a useful chapter but no mention is made of the possibility of using resistant varieties and stem builders to reduce the incidence of Bacterial Canker. The author makes suggestions for the selection of varieties both for orchard and garden culture. The chapter on jam-making, canning, bottling, drying and juice manufacture quotes the best available information, but the space given to the chemistry of the plum seems somewhat out of place, and figures given in Chapter VIII and again in Chapter IX for the percentage of sugar in greengages, Victoria and culinary plums, although quoted from the same source, do not seem to agree.

The chapter on Plum Characters in which the author attempts a new system for the identification of varieties, is separated unfortunately from Part II by a chapter on the choice of varieties for growing under varied conditions. It would have been better placed immediately before the detailed variety descriptions included in Part II.

The work of Hogg* and others on the classification of plums is somewhat discounted by the author but it is doubtful if the system put forward by Dr. Taylor whereby plums are identified by the fruits alone will be any better. However, his suggestions, although not with a systematic botanical approach, should help in the ever interesting and often baffling job of naming varieties correctly from fruit characters alone. Under the heading of colour, an error occurs in the tables where Severn Cross, a pale golden yellow plum, appears under both green and red plums.

Part II is devoted mainly to descriptions of some 120 varieties. These are in very general terms and show little advance on previous descriptions; some varieties appear as little else than a name. The origin of the variety Marjorie's Seedling is not given. This valuable late culinary plum was the result of a chance seedling grown on the farm of Mr. George Layley, Beenham, Berkshire, and named Marjorie after Mr. Layley's daughter. Scions of this plum were first collected and sent to the R.H.S. in the early 1920's by Mr. A. E. Barnes, Horticultural Instructor for the county of Berkshire. The variety Thames Cross, raised by Mr. G. T. Spinks of Long Ashton, is stated to be similar to Teme Cross but ripening a fortnight earlier. Teme Cross, however, ripens in August and it is the variety Severn Cross which is meant. This part of the book is, however, most useful. Many varieties hitherto not known to the ordinary reader are included and there are 32 coloured plates, superbly reproduced, which not only delight the eye but are also a valuable source of reference.

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* Hogg's name is also omitted from the Bibliography on p. 99.

The Plums of England will be a useful addition to the fruitgrower's bookshelf. Who now will deal with the pears ?

E. W. HOBBIS.

INDEX TO HORTICULTURAL ABSTRACTS. Volumes XI-XV (1941-1945). Compiled by D. Akenhead. (East Malling: Commonwealth Bureau of Horticulture and Plantation Crops. 1949. Price 35s.)

" The one aim of this work," writes the compiler, " is to make readily available to the English-speaking horticultural worker the information contained in *Horticultural Abstracts*, Volumes XI-XV." It will do precisely that, and strictly by way of review there is no more to be said. The appearance of a cumulative index, however, is a reminder of the debt that horticultural science owes to the abstracting services of the Commonwealth Agricultural Bureaux collectively and the Bureau located at East Malling in particular.

The first cumulative index to *Horticultural Abstracts* covered ten years and ten volumes (I-X), and referred to abstracts and notes on 9,549 original publications. The present index covers only five years and five volumes, but refers to 10,289 original publications—the figures are taken from a very informative " subject analysis of abstracts " presented with the preface. The difference is doubtless due in part to the tireless enthusiasm with which the Bureau casts its net ever wider and wider as more and more obscure publications are brought to its notice ; but mainly it must be symptomatic of the accelerating output of scientific literature generally.

Distilling the essence from more than 40 papers, volumes and reports a week, many of them in foreign languages, must often become a tedious labour for which thanks are due to those who do it so well. The indexing and cross-referencing of the abstracts is still more laborious, but it is the necessary completion of the service rendered. Now, thanks to Mr. Akenhead, the horticultural worker can in a few minutes trace information on carrots or cocoa, gooseberries or guavas ; and, if we know our Bureau, he can be fairly confident that he is not missing much of significance written on his subject in the years 1941-45. Within the field of horticulture and plantation crops the rising flood of literature seems to be reasonably under control.

E.E.C.

THE FRUIT YEAR BOOK, 1949. (London: Royal Horticultural Society. 1949. Pp. 167. Price 8s. 6d.)

The third *Fruit Year Book*, dedicated to Mr. Edward A. L. Laxton and opening with an appreciation of his work in fruit breeding, maintains the standard of its predecessors and needs no higher praise. Variety is its charm ; and among twenty-five articles, ranging from the origin of the garden plum (M. B. Crane) to preserving fruit for quality (Alice Crang) there is something for everybody rightmindedly interested in fruitgrowing.

If the reader's first interest is in his own garden he may start with discussions of cultural systems for apples and pears (E. W. Hobbis), the pruning of bush apples (A. P. Preston), rindgrafting technique (H. Denham), or two severely practical articles on spraying and spray equipment emanating from East Malling (H. B. S. Montgomery) and Long Ashton (H. G. H. Kearns and N. G. Morgan). Two accounts of fruitgrowing in Scotland—apples and pears by D. L. Storrie, and soft fruits by R. D. Reid—will assist him if his garden is in the north and interest him wherever it is.

Other people's problems, and gardens overseas, are mentioned by H. M. Tydeman, who went round Scandinavia in search of new fruits ; F. C. H. Gayner, who writes about the amateur in South Africa ; F. M. Read on fruitgrowing in Victoria, Australia ; J. P. Hudson on New Zealand orchards ; and R. C. Palmer on fruitgrowing under irrigation in the sunny Okanagan valley of British Columbia. Photographs add to the enjoyment of the world tour thus provided.

General information of another kind is to be found in Professor Barker's explanation of what a cider apple is ; L. C. Luckwill's brief account of the many roles of hormones in the fruit garden ; and R. Barrington Brock's notes on viticulture in Surrey. The story of the Big Gooseberry cult is a quaint bit of horticultural history amusingly told by Gavin Brown.

Notes on Fruit Group discussions of " Cherries for the garden ", " Plums ", and " Late-keeping apples " are followed by a useful short bibliography of original researches published by the fruit Research Stations during the year and a number of book reviews. The colour plate this year is of strawberry varieties and is accompanied by notes by W. S. Rogers.

E.E.C.

LABORATORY METHODS FOR WORK WITH PLANT AND SOIL NEMATODES. By T. Goodey. Ministry of Agriculture and Fisheries Technical Bulletin No. 2. (London : His Majesty's Stationery Office, 1949. Pp. 19. Price 9d. net.)

Dr. Goodey has gathered together in a very practical way much hitherto scattered information on the collection and laboratory processing of the group of nematodes commonly known as eelworms. Several of these methods have been developed in his department at Rothamsted while others are of American origin. Of recent years eelworms have been handled by an increasing number of biologists, many of whom will find these techniques invaluable where those normally applicable to arthropods have failed.

R.S.P.

ONIONS AND RELATED CROPS. Bulletin No. 69 of the Ministry of Agriculture and Fisheries. (London : His Majesty's Stationery Office, 1949. Pp. 38. Price 1s. 3d. net.)

A revision of a bulletin first published in 1934. With the setting up of the Ministry's National Agricultural Advisory Service, responsibility for the information on cultivation in the Ministry's horticultural publications now rests with the Advisory

Specialists who are attached to the provincial headquarters of the Service. Revision of this bulletin was carried out by the Vegetable Group, under the Chairmanship of Mr. F. A. Secrett, C.B.E., F.L.S., V.M.H. The related crops are shallots, garlic and leeks. There are 12 photographs, six of them illustrating the production of onions from sets.

PEAS. Bulletin No. 81 of the Ministry of Agriculture and Fisheries. (London: His Majesty's Stationery Office, 1949. Pp. 22. Price 1s. 3d. net.)

Another useful revision, the first edition having been published in 1935. Illustrated by 19 photographs of varieties and 7 showing cultural and harvesting operations.

STRAWBERRIES. Bulletin No. 95 of the Ministry of Agriculture and Fisheries. (London: His Majesty's Stationery Office, 1949. Pp. 38. Price 2s. net.)

The fourth edition of a bulletin first published in 1937, revised by the Fruit Group of Advisory Specialists of the National Agricultural Advisory Service under the Chairmanship of Professor T. Wallace. Well illustrated (31 figures including 26 photographs) and includes a key for the identification of ten of the commonest varieties of strawberries.

BEE-HIVES. Bulletin No. 144 of the Ministry of Agriculture and Fisheries. (London: His Majesty's Stationery Office, 1949. Pp. 20. Price 9d. net.)

A new bulletin, written by Dr. C. G. Butler of Rothamsted Experimental Station and intended to provide an authoritative and up-to-date account of the types and qualities of bee-hive at present available. Illustrated by 4 half-tone plates.

BETTER GLASSHOUSE CROPS. By W. J. C. Lawrence. (London: George Allen and Unwin, Ltd. 1949. Pp. 57. Price 5s.)

It is stated in the preface to this slim little volume that the separate chapters first appeared in *The Grower* as a series of monthly articles. They deal with choice and preparation of loam, soil sterilization, seed and potting composts, the sterilizing and mixing shed, using the right compost, transplanting, tomato raising and lettuce raising. The text is an admirable popular summary of recommendations resulting from the author's well-known research.

ROSES. By Bertram Park. (London: Sir Isaac Pitman & Sons, Ltd. 1949. Pp. 141, illus. Price 10s. 6d. net.)

Chapters deal with the planning and preparation of a rose-garden, the various types of roses, planting, pruning, manures and manuring, pests and diseases, rose-shows.

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